Supplemental Figures

Genetic associations of T cell cancer immune response-related genes with T cell phenotypes and clinical outcomes of early-stage lung cancer

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T cell cancer immune response genes

Identified 312 genes in 25 pathways

Discovery Phase (MD Anderson)
Genotyped 536 patients from an ongoing GWAS study

Validation Phase (MD Anderson)
Genotyped 405 patients using OncoArray chip

Meta-analysis
14 SNPs in 9 genes validated

Cumulative analysis
P<0.001 in both cohorts (MD Anderson)

Stratified analysis
Surgery-only and Surgery-plus-chemotherapy

Genotype-Phenotype Correlation and Functional Analysis

T cell degranulation & cytotoxicity assays
T cells from PMBCs of 38 healthy donors

Immune gene expression profile
18-Gene panel of T cell function indicating T cell activation

eQTL analysis
6 SNPs in 5 genes associated with gene expression (HaploReg)

Tumor/normal tissue analysis
Expression of 3 genes were significantly different (TCGA)

Supplemental Figure S1: Schematic flowchart of study design
Supplemental Figure S2. Gating strategy for T cell CD107a degranulation assay

A gating strategy for flow cytometry analysis of T cell degranulation assay is shown. (A) Dead cells were excluded by SYTOX Blue staining. Single cells were obtained by gating on FSC-A versus FSC-H, followed by SSC-A versus SSC-H. (B) Representative controls of CD107a degranulation assay. CD8+CD107a+ T cells were analyzed and counted under unstimulated (PBS) and stimulated (CD3 antibody [OKT3]). Data were analyzed using FlowJo (v.10.0.8) software.
Supplemental Figure S3. Workflow of in vitro T cell killing assay.
Schematic flowchart of the image-based phenotypic assay to assess the level of T cell cytotoxicity in peripheral blood mononuclear cells (PBMCs) obtained from healthy low (0 or 1) and high (≥3) unfavorable genotype (UFG) carriers.
Supplemental Figure S4. Gating strategy and time course of T cell killing by effector-to-target cell ratio.

(A) Single leukocytes were obtained by gating on FSC-A versus FSC-H, followed by SSC-A versus SSC-H. Dead cells were excluded by SYTOX Blue staining, and CD3+ T cells were identified and sorted. Data were analyzed using FlowJo (v.10.0.8) software. (B) The time course of T cell cytotoxicity by effector-to-target cell (E:T) ratios for A549 and H460 cell lines indicates a dose-response trend.
Supplemental Figure S5. Kaplan-Meier analyses of recurrence-free survival (RFS) for patients with early-stage NSCLC by genotypes of SNPs predictive of recurrence risk in the discovery and validation phases.

RFS of patients in the discovery and validation sets by genotypes of SYK rs10761395 (A and B, respectively), TGRA rs7155927 (C and D), CD4 rs3782736 (E and F), TRB rs1573618 (G and H), and PDCD1LG2 rs7854413 (I and J). For all genetic models, blue line includes homozygous wildtype allele carriers; red line includes homozygous variant allele carriers; and green line indicates heterozygous allele carriers. MST, median recurrence-free survival time.
Supplemental Figure S6. Kaplan-Meier analyses of overall survival for patients with early-stage NSCLC by genotypes of SNPs predictive of death risk in the discovery and validation phases.

Overall survival of patients in the discovery and validation sets by genotypes of CUL1:rs122571 (A and B), GRB2:959260 (C and D), CUL1:rs243538 (E and F), GRB2:rs4789182 (G and H), TRB1:1573618 (I and J), and JAK1:rs4915675 (K and L). For all genetic models, blue line includes homozygous wildtype allele carriers; red line includes homozygous variant allele carriers; and green line indicates heterozygous allele carriers. MST, median survival time.
Supplemental Figure S7: Kaplan Meier analyses of combined unfavorable genotypes (UFGs) associated with recurrence and death risks

(A and B) Recurrence-free survival by number of UFGs for 7 SNPs associated with NSCLC recurrence in the discovery and validation sets. SNPs associated with recurrence are TRB: rs1964986, IL2RB: rs3218339, SYK: rs10761395, TRA: rs7155927, CD4: rs3782736, TRB: rs1573618, and PDCD1LG2: rs7854413. (C and D) Overall survival by number of UFGs for 7 SNPs associated with death risk in the discovery and validation sets. SNPs associated with survival are IDO1: rs10108662, CUL1: rs122571, GRB2: rs959260, CUL1: rs243538, GRB2: rs4789182, TRB: rs1573618, JAK1: rs4915675.
Supplemental Figure S8. Expressions of candidate T cell cancer immune response genes in NSCLC tumor and normal tissues from TCGA data.

The expressions of the identified eQTL SNP–located genes in tumor (N=1016) and normal tissues (N=110) were assessed in NSCLC (lung adenocarcinoma and squamous cell carcinoma) datasets using level 3 RSEM normalized mRNA sequencing data from The Cancer Genome Atlas (TCGA). *GRB2 and JAK1 expressions were significantly lower in tumor tissues (P<0.01), whereas PSMD3 expression was significantly higher in tumor tissues (P<0.01). GSK3B and IDO1 expression in tumor and normal tissues did not differ significantly with the latter showing marginal significance (P=0.11). * P<0.05.
Supplemental Figure S9. Unfavorable genotypes (UFGs) in the T cell cancer immune response and T cell cytotoxicity in vitro.

We used an image-based in vitro cell-based assay to assess T cell cytotoxicity against NSCLC cells. Representative images of the two NSCLC cell lines used as cell targets (A459 [top row] and H460 [bottom row]) are shown. Green fluorescence indicates live cancer cells; red fluorescence indicates dead cancer cells; and blue fluorescence indicates T cells isolated from peripheral blood of human subjects. NSCLC cells without the addition of T cells were used as negative control (first column), and 70% ethanol–treated cancer cells were used as positive control (fourth column). Representative images of T cell cytotoxicity from high-risk group (≥3 UFGs) (second column) and low-risk group (0 or 1 UFG) (third column) are displayed.