

Supplemental Tables

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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Supplemental Table S1. Summary of the pathways and gene counts in the T cell cancer immune response

T cell cancer immune response step	Pathway involved	Gene count
Cancer antigen processing and presentation	MHC-I antigen presentation	76
	MHC-II antigen presentation	11
	CD40 pathway	40
	IL-10 pathway	8
	IFN-alpha pathway	9
	IL-4 signaling pathway	12
	T helper cell surface molecules	12
	Th1/Th2 differentiation	20
	TLR pathway	106
T cell priming and activation	CD28 co-stimulation	88
	4-1BB-dependent immune response	11
	OX40 pathway	3
	IL-2 pathway	17
	IL-12 pathway	17
	T cell surface markers and ligands	22
	PD-1 pathway	27
T cell trafficking	Chemokine pathway	68
	CX3CL1, CXCL9, CXCL10, CCL5*	5
T cell infiltration	ITGAL1, ICAM1, SELE, TGFB1, TGFBR2, VEGF, EMOES*	6
T cell recognition	TCR pathway	171
T cell cytotoxicity	IFN-gamma pathway	36
	CTL pathway	15
	T cytotoxic cell surface marker	12
	Inhibitory genes FOXP3, IDO1*	2
	T cell surface markers and ligands	22
Total		815
Candidate gene list [†]		314
Final gene list [‡]		280

* Based on immune gene panels from Nanostring and HTG and on Singer M et al., Cell 2016;166:1500-1511, Chen DS et al., Immunity 2013;39:1-10, and Mariathasan S et al., Nature 2018;554:544-548.

[†] Genes involved in at least two pathways or mentioned in two databases were selected for the candidate gene list.

[‡] Genes included in the GWAS panel and after quality control.

Supplemental Table S2. Genotype and phenotype characteristics of donors in T cell degranulation and cytolytic phenotypic assays

Donor Pair	Genotype information							CD107a+CD8+ T cells (%)		*Cytotoxicity (mean(SD))			
	‡Low risk (0~1 UFG)	‡High risk (3~4 UFGs)								A549		H460	
	rs4688054	rs959260	rs8080546	rs4789182	rs4915675	rs10108662	rs4688054	Low risk	High risk	Low risk	High risk	Low risk	High risk
1	No	Yes	Yes	Yes	No	Yes	No	3.4	2.3	0.546(0.139)	0.676(0.160)	0.671(0.158)	0.657(0.155)
2	Yes	Yes	Yes	Yes	No	No	No	3.2	2.7	0.609(0.174)	0.531(0.130)	NA	NA
3	No	Yes	Yes	Yes	No	No	No	1.4	4.2	0.422(0.145)	0.256(0.099)	NA	NA
4	No	Yes	Yes	Yes	No	No	No	8.3	12.1	0.421(0.103)	0.563(0.135)	0.473(0.088)	0.623(0.165)
5	Yes	Yes	Yes	Yes	No	No	No	17.6	1.2	0.380(0.117)	0.227(0.081)	0.475(0.122)	0.274(0.092)
6	No	Yes	Yes	Yes	No	Yes	No	1	2.9	0.383(0.114)	0.267(0.085)	NA	NA
7	No	No	Yes	Yes	Yes	No	Yes	3.5	1.3	0.629(0.252)	0.520(0.214)	0.666(0.192)	0.600(0.170)
8	Yes	Yes	Yes	Yes	Yes	No	No	1.7	1.3	0.467(0.112)	0.357(0.103)	0.513(0.107)	0.400(0.101)
9	No	Yes	Yes	Yes	No	No	No	11.6	4.1	NA	NA	NA	NA
10	No	Yes	Yes	Yes	No	Yes	No	0.1	2.4	NA	NA	NA	NA
11	No	Yes	Yes	Yes	Yes	No	No	12.1	11	NA	NA	NA	NA
12	No	Yes	Yes	Yes	No	Yes	No	7.6	6.6	NA	NA	NA	NA
13	No	Yes	Yes	Yes	No	No	Yes	12.4	7.4	NA	NA	NA	NA
14	No	Yes	Yes	Yes	No	No	Yes	5.2	3.7	NA	NA	NA	NA
15	No	No	Yes	Yes	Yes	No	No	10.5	1.4	NA	NA	NA	NA
16	No	Yes	Yes	Yes	No	No	No	10.1	5.3	NA	NA	NA	NA
17	No	Yes	Yes	Yes	No	No	Yes	6.3	5.5	NA	NA	NA	NA
18	No	No	Yes	No	Yes	No	Yes	15.9	12.5	NA	NA	NA	NA
19	No	Yes	Yes	Yes	No	Yes	No	2	2	NA	NA	NA	NA

Abbreviations: UFG, unfavorable genotype; NA, data not available due to insufficient T cells or sample stocks.

‡ Low-risk group subject has at most 1 UFG (rs4688054); high-risk group subject has 3 or more UFGs.

* Cytotoxicity level is the mean inhibitory rate of all time points and replicates.

NA: PBMC samples storage is not enough for this assay

Supplemental Table S3. T cell functional gene panel and TaqMan assays

Gene	Full Name	Assay ID	Catalog No.
T cell cytotoxicity-related genes			
<i>IL2</i>	Interleukin 2 (IL2)	Hs00174114_m1	4331182
<i>IFNG</i>	Interferon gamma (IFNG)	Hs00989291_m1	4331182
<i>PRF1</i>	Perforin 1 (PRF1)	Hs00169473_m1	4331182
<i>GZMB</i>	Granzyme B (GZMB)	Hs00188051_m1	4331182
<i>TNFA</i>	Tumor necrosis factor alpha (TNFA)	Hs00174128_m1	4331182
T cell inhibitory genes			
<i>FOXP3</i>	Leptin (LEP)	Hs01085834_m1	4331182
<i>IL4</i>	Interleukin 4 (IL4)	Hs00174122_m1	4331182
T cell trafficking gene			
<i>EOMES</i>	Eomesodermin (EOMES)	Hs00172872_m1	4331182
Immune checkpoint genes			
<i>HAVCR2</i>	Hepatitis A virus cellular receptor 2 (HAVCR2)	Hs00958618_m1	4331182
<i>PDCD1</i>	Programmed cell death 1 (PDCD1)	Hs01550088_m1	4331182
<i>CTLA4</i>	Cytotoxic T-lymphocyte associated protein 4 (CTLA4)	Hs00175480_m1	4331182
<i>LAG3</i>	Lymphocyte activating 3 (LAG3)	Hs00958444_g1	4351372
<i>CD137</i>	TNF receptor superfamily member 9 (TNFRSF9)	Hs00155512_m1	4331182
<i>VISTA</i>	V-domain Ig suppressor of T cell activation (VISTA)	Hs00735289_m1	4331182
<i>ICOS</i>	Interleukin 1 beta (IL1B)	Hs00359999_m1	4331182
<i>IDO1</i>	Indoleamine 2,3-dioxygenase 1 (IDO1)	Hs00984148_m1	4331182
Housekeeping and lineage genes			
<i>CD4</i>	CD4 molecule (CD4)	Hs01058407_m1	4331182
<i>CD8</i>	CD8 molecule (CD8)	Hs00233520_m1	4331182
<i>CD3E</i>	CD3e molecule (CD3E)	Hs01062241_m1	4331182
<i>GAPDH</i>	Glyceraldehyde-3-phosphate dehydrogenase (GAPDH)	Hs01548420_m1	4331182

Supplemental Table S4: The details of chemotherapy information

Chemo-regimens	Freq.	Percent
Carboplatin + paclitaxel	93	9.88
Carboplatin only	32	3.4
Carboplatin + Docetaxel	26	2.76
Cisplatin only	17	1.81
Docetaxel + Cisplatin	43	4.57
Others*	40	4.25
No chemotherapy	658	69.93
Unknown	32	3.4
Total	941	100

*Others indicates chemo-regimens other than above, like carboplatin plus gemcitabine/etoposide, etc.

Supplemental Table S5. Predictive single-nucleotide polymorphisms(SNPs) for surgery alone and surgery and adjuvant chemotherapy (Without adjustment of smoking status)

Gene SNP by outcome	Location	Model	Surgery only		Surgery plus chemotherapy		Group¶
			HR (95%CI)†	P	HR (95%CI)†	P	
Recurrence							
VAV2: rs491220‡	3'UTR	REC	2.12(1.29-3.49)	3.09E-03	0.33 (0.12-0.89)	2.86E-02	1
IFNGR2: rs1059293	3'UTR	REC	0.46 (0.22-0.96)	3.74E-02	2.88 (1.31-6.32)	8.21E-03	2
PTPRC: rs2359952	Intron	REC	2.03 (1.14-3.61)	1.66E-02	4.53 (1.42-14.4)	1.06E-02	3
TRA: rs2049787	3'UTR	REC	1.79 (0.97-3.31)	6.47E-02	4.73 (1.91-11.8)	8.10E-04	3
NRAS: rs10489525	Intron	DOM	0.63 (0.40-0.97)	3.79E-02	0.46 (0.22-0.99)	4.70E-02	3
Survival							
TRB: rs10231513	Intron	DOM	2.00 (1.30-3.10)	1.80E-03	0.35(0.15-0.79)	1.19E-02	1
STAT4: rs3024896	Intron	DOM	1.40 (0.98-2.01)	6.72E-02	0.27 (0.08-0.95)	4.06E-02	1
PTK2B: rs2322718	Intron	REC	1.57 (1.03-2.39)	3.61E-02	0.16 (0.04-0.70)	1.52E-02	1
CUL1: rs243511‡	Intron	ADD	0.44 (0.26-0.76)	2.97E-03	2.32 (0.95-5.65)	6.42E-02	2
MAP3K1: rs12655019	Intron	DOM	0.71 (0.56-0.91)	5.89E-03	2.17 (1.10-4.28)	2.58E-02	2
VAV2: rs2797826	Intron	DOM	0.71 (0.49-1.03)	7.43E-02	0.38 (0.17-0.85)	1.82E-02	3

Abbreviations: HR: hazard ratio, CI, confidence interval; REC, recessive; ADD, additive; DOM, dominant. a: HR was adjusted by age, gender, smoking status and stage, the HR of meta-analysis based on fixed effects model in two phases.

†: The HR was adjusted for age, gender and disease stage

‡: rs521446 is linked with rs491220, $R^2=1$, rs243511 is linked with rs243519, $R^2=1$, thus the data of them were not shown.

¶: Group 1 indicated SNP associate with increased recurrence/death risk in surgery only patients, but reduced risk in surgery plus chemotherapy patients, group 2 indicated SNP associated with reduced recurrence/death risk in surgery only patients, but increased risk in surgery plus chemotherapy, group 3 indicated SNP associated with increased recurrence/death risk in both treatment arms, while group 4 indicated SNP associated with reduced risk in both treatment arms.

Supplemental Table S6. Cumulative effects of unfavorable genotypes on recurrence and death risks in early-stage NSCLC patients

Discovery set						Validation set				
No. of UFGs	Rec N(%)	No Rec N(%)	HR (95%CI)*	P	Log-rank P	Rec N(%)	No Rec N(%)	HR (95%CI)*	P	Log-rank P
0-2	28(20.90)	106(79.10)	1(reference)			20(19.05)	85(80.95)	1(reference)		
3	67(34.90)	125(65.10)	2.00(1.21-3.32)	7.02E-03		38(25.33)	112(74.67)	2.02(1.02-4.00)	4.48E-02	
4-6	82(39.61)	125(60.39)	2.86(1.76-4.64)	2.15E-05		60(41.10)	86(58.90)	3.88(1.99-7.56)	6.73E-05	
			1.63(1.31-2.04)	1.42E-05	3.84E-04			1.96(1.45-2.64)	1.25E-05	2.07E-05
No. of UFGs	Alive N(%)	Dead N(%)	HR (95%CI)*	P	Log-rank P	Alive N(%)	Dead N(%)	HR (95%CI)*	P	Log-rank P
0	24(30.38)	55(69.62)	1(reference)			10(17.86)	46(82.14)	1(reference)		
1-3	127(41.50)	179(58.50)	1.69(1.09-2.63)	1.99E-02		48(22.12)	169(77.88)	1.68(0.84-3.37)	1.41E-01	
4-7	75(50.68)	73(49.32)	2.37(1.48-3.79)	3.35E-04		51(39.53)	78(60.47)	3.73(1.85-7.54)	2.46E-04	
			1.50(1.21-1.85)	2.01E-04	7.72E-03			2.04(1.50-2.80)	7.39E-06	1.07E-04

Abbreviations: NSCLC, non-small cell lung cancer; UFG, Unfavorable genotype; Rec, recurrence; CI, confidence interval.

*Adjusted according to gender, age, smoking status, tumor stage, performance status and treatment.

Rs1964986, rs3218339, rs10761395, rs7155927, rs3782736, rs7155927 and rs7854413 were included in the recurrence analysis; rs959260, rs4789182, rs4915675, rs243538, rs122571, rs1573618 and rs10108662 were included in the survival analysis.

Supplemental Table S7. Subgroup analysis of SNPs associated with recurrence and survival in patients with early-stage NSCLC stratified by treatment

Gene-SNP by outcome	Model	Discovery		Validation		Meta-analysis*		
		HR (95%CI)†	P	HR (95%CI)†	P	HR (95%CI)†	P	P_het
Recurrence								
Surgery only								
<i>PRKCA</i> : rs16960070 ‡	DOM	2.44 (1.50-3.97)	3.10E-04	2.27 (1.09-4.73)	2.80E-02	2.39 (1.59-3.58)	2.62E-05	0.872
<i>TRB</i> : rs1964986 ‡	DOM	0.63 (0.39-1.00)	4.82E-02	0.45 (0.21-0.97)	4.01E-02	0.57 (0.38-0.86)	6.72E-03	0.463
Surgery plus chemotherapy								
<i>TRB</i> : rs1964986 ‡	REC	2.36 (1.12-4.99)	2.44E-02	3.89 (1.57-9.66)	3.35E-03	2.89 (1.62-5.14)	3.16E-04	0.405
<i>TRB</i> : rs2367486	REC	2.14 (1.06-4.35)	3.49E-02	2.93 (1.15-7.45)	2.42E-02	2.40 (1.37-4.21)	2.33E-03	0.599
Survival								
Surgery only								
<i>PSMD3</i> :rs8080546 ‡	DOM	1.75 (1.19-2.57)	4.53E-03	2.13 (1.19-3.81)	1.05E-02	1.86 (1.35-2.56)	1.56E-04	0.581
<i>TRB</i> : rs1964986 ‡	DOM	0.56 (0.39-0.81)	1.97E-03	0.59 (0.35-0.99)	4.76E-02	0.57 (0.42-0.77)	2.26E-04	0.872
<i>CUL1</i> : rs243511 ‡	ADD	0.76 (0.58-1.00)	4.89E-02	0.67 (0.47-0.96)	2.72E-02	0.73 (0.58-0.90)	3.69E-03	0.582
Surgery plus chemotherapy								
<i>IKBKB</i> : rs5029748	ADD	1.72 (1.11-2.66)	1.52E-02	2.10 (1.11-3.96)	2.17E-02	1.83 (1.28-2.63)	9.68E-04	0.612
<i>NR1I2</i> : rs3732357	ADD	1.74 (1.07-2.81)	2.52E-02	1.84 (1.05-3.22)	3.37E-02	1.78 (1.24-2.57)	1.96E-03	0.882
<i>RELA</i> : rs7101916	DOM	0.48 (0.24-0.97)	4.17E-02	0.16 (0.04-0.77)	2.18E-02	0.39 (0.21-0.74)	3.73E-03	0.188
<i>GSK3B</i> : rs4688054 ‡	ADD	1.57 (1.00-2.45)	4.60E-02	2.38 (1.12-5.04)	2.39E-02	1.75 (1.19-2.57)	4.35E-03	0.352
<i>RELA</i> : rs11227247 ‡	DOM	0.48 (0.24-0.97)	4.17E-02	0.16 (0.03-0.77)	2.18E-02	0.40 (0.21-0.77)	5.65E-03	0.223
<i>PTPRC</i> : rs2038926 ‡	REC	0.34 (0.12-0.95)	3.88E-02	0.10 (0.01-0.99)	4.88E-02	0.28 (0.11-0.71)	7.58E-03	0.341

Abbreviations: NSCLC, non-small cell lung cancer; SNP, single-nucleotide polymorphism; HR, hazard ratio; CI, confidence interval; REC, recessive; DOM, dominant; ADD, additive; P het, P test for heterogeneity.

* The meta-analysis was based on a fixed effect model.

† HR was adjusted for gender, age, smoking status, tumor stage, performance status and treatment.

‡ In the validation phase, data for linked SNPs ($r^2 > 0.8$) were shown. Specifically, rs9896191 replaced rs16960070; rs10273639 replaced rs1964986; rs2827 replaced rs8080546; rs243519 replaced rs243511; rs3732359 replaced rs4688054; rs7101916 replaced rs11227247; and rs6686725 replaced rs2038926.

Supplemental Table S8. Predictive single-nucleotide polymorphisms(SNPs) for surgery alone and surgery and adjuvant chemotherapy (Adjusted with chemotherapy type)

Gene SNP by outcome	Location	Model	Surgery only		Surgery plus chemotherapy		Group¶
			HR (95%CI)†	P	HR (95%CI)†	P	
Recurrence							
VAV2: rs491220‡	3'UTR	REC	2.12(1.29-3.50)	3.06E-03	0.29 (0.10-0.85)	2.30E-02	1
IFNGR2: rs1059293	3'UTR	REC	0.45 (0.22-0.94)	3.42E-02	3.94 (1.65-9.39)	2.01E-03	2
PTPRC: rs2359952	Intron	REC	1.92 (1.08-3.45)	2.72E-02	8.60 (2.25-32.8)	1.63E-03	3
TRA: rs2049787	3'UTR	REC	1.88 (1.01-3.49)	4.57E-02	4.39 (1.61-11.9)	3.74E-03	3
NRAS: rs10489525	Intron	DOM	0.59 (0.38-0.93)	2.15E-02	0.43 (0.19-0.98)	4.42E-02	3
Survival							
TRB: rs10231513	Intron	DOM	2.02 (1.30-3.14)	1.66E-03	0.31 (0.13-0.75)	8.79E-03	1
STAT4: rs3024896	Intron	DOM	1.46 (1.01-2.10)	4.17E-02	0.28 (0.08-0.97)	4.54E-02	1
PTK2B: rs2322718	Intron	REC	1.53 (1.01-2.33)	4.62E-02	0.16 (0.04-0.70)	1.48E-02	1
CUL1: rs243511‡	Intron	ADD	0.46 (0.27-0.79)	4.96E-03	2.69 (1.03-7.05)	4.41E-02	2
MAP3K1: rs12655019	Intron	DOM	0.72 (0.56-0.92)	7.62E-03	1.96 (0.98-3.90)	5.64E-02	2
VAV2: rs2797826	Intron	DOM	0.68 (0.47-0.99)	4.64E-02	0.38 (0.17-0.86)	2.04E-02	3

Abbreviations: HR: hazard ratio, CI, confidence interval; REC, recessive; ADD, additive; DOM, dominant. a: HR was adjusted by age, gender, smoking status and stage, the HR of meta-analysis based on fixed effects model in two phases.

†: The HR was adjusted for age, gender, smoking status, chemotherapy type and disease stage

‡: rs521446 is linked with rs491220, $R^2=1$, rs243511 is linked with rs243519, $R^2=1$, thus the data of them were not shown.

¶: Group 1 indicated SNP associate with increased recurrence/death risk in surgery only patients, but reduced risk in surgery plus chemotherapy patients, group 2 indicated SNP associated with reduced recurrence/death risk in surgery only patients, but increased risk in surgery plus chemotherapy, group 3 indicated SNP associated with increased recurrence/death risk in both treatment arms, while group 4 indicated SNP associated with reduced risk in both treatment arms.

Supplemental Table S9. Cumulative effect of unfavorable genotypes predicting opposite effects on risks of recurrence and death in surgery-only and surgery-plus-chemotherapy NSCLC patients

Recurrence	No. of	Surgery only					*Surgery plus chemotherapy					
		Genotype	UFGs	No rec, N(%)	Rec, N(%)	HR (95%CI)†	P	Log-rank P	No rec, N(%)	Rec, N(%)	HR (95%CI)†	P
Low risk group	0		56(84.85)	10(15.15)	1(reference)			14(58.33)	10(41.67)	1(reference)		
IM risk group	1		202(72.92)	75(27.08)	1.85(0.83-4.09)	0.130		50(73.53)	18(26.47)	0.39(0.17-0.91)	0.029	
High risk group	2		38(62.30)	23(37.70)	3.73(1.56-8.93)	3.16E-03	4.29E-03	24(82.76)	5(17.24)	0.14(0.04-0.50)	2.57E-03	4.95E-02
Survival	No. of	Surgery only					*Surgery plus chemotherapy					
Genotype	UFGs	Alive, N(%)	Dead, N(%)	HR (95%CI)†	P	Log-rank P	Alive, N(%)	Dead, N(%)	HR (95%CI)†	P	Log-rank P	
Low risk group	0~1	55(85.94)	9(14.06)	1(reference)			10(43.48)	13(56.52)	1(reference)			
IM risk group	2	98(75.38)	32(24.62)	2.58(1.22-5.46)	0.013		35(81.40)	8(18.60)	0.27(0.10-0.70)	0.007		
High risk group	3~5	120(62.18)	73(37.82)	3.85(1.91-7.77)	1.70E-04	3.91E-04	45(88.24)	6(11.76)	0.08(0.03-0.26)	1.44E-05	1.82E-05	

Abbreviation: Rec, recurrence; UFG: unfavorable genotypes defined in surgery-only patients, IM: intermediate

* Chemotherapy includes adjuvant chemotherapy and chemo-radiation therapy.

† Adjusted according to gender, age, smoking status, tumor stage, performance status and treatment;

SNP included in analysis for recurrence risk: rs491220, rs1059293; for death risk: rs10231513, rs3024896, rs2322718, rs243511, rs12655019.

Supplement Table S10. Significant SNPs associated with gene expression in blood leukocytes in cis-eQTL analysis

SNP	Gene	Chr.	Position	SNP Alleles	Minor Allele	*Z-score	*FDR	*P-value	**Z-score	**Correlation Coefficient	**P-value
rs959260	GRB2	17	70881017	G/A	G	-13.85	0	1.32E-43	-9.73484	-0.23;-0.36	2.14E-22
rs4915675	JAK1	1	65200064	G/A	G	-30.52	0	9.81E-198	-14.8838	-0.4	4.20E-50
rs4789182	GRB2	17	70886540	A/G	G	-3.87	0	0.0421	-11.0063	-0.25;-0.43	3.57E-28
rs10108662	IDO1	8	39899146	C/A	A	NA	NA	NA	5.9265	0.38	3.10E-09
rs4688054	GSK3B	3	1.21E+08	A/G	G	NA	NA	NA	5.58	0.14;0.18	NA
rs8080546	PSMD3	17	35400492	C/A	A	12.43	0	1.74E-35	NA	NA	NA

All analyses were performed based on peripheral blood leukocyte data.

* Data from Nat Genet. 2013;45(10):1238-1243; ** data from PLoS Genet. 2011; 7(8): e1002197.

NA, data not available.

Supplemental Table S11. Genotypes of eQTL significant SNPs

SNP	Gene Name	Unfavorable genotype	Favorable genotype	Unfavorable allele	Model	Ref Allele
rs959260	GRB2	GG+GA	AA	Variant	DOM	A
rs4915675	JAK1	GG	AA+AG	Variant	REC	A
rs8080546	PSMD3	AA+AC	CC	Variant	DOM	C
rs4789182	GRB2	GG+GA	AA	Variant	DOM	A
rs10108662	IDO1	AA	AC+CC	Variant	REC	C
rs4688054	GSK3B	GG+GA	AA	Variant	ADD	A

Abbreviations: eQTL, expression quantitative trait loci; DOM, dominant; REC, recessive; ADD, additive.

