

Prevalent Homozygous Deletions of Type I Interferon and Defensin Genes in Human Cancers Associate with Immunotherapy Resistance



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Abstract

Purpose: Homozygous deletions play important roles in carcinogenesis. The genome-wide screening for homozygously deleted genes in many different cancer types with a large number of patient specimens representing the tumor heterogeneity has not been done.

Experimental Design: We performed integrative analyses of the copy-number profiles of 10,759 patients across 31 cancer types from The Cancer Genome Atlas project.

Results: We found that the type-I interferon, α -, and β -defensin genes were homozygously deleted in 19 cancer types with high frequencies (7%–31%, median = 12%; interquartile range = 10%–16.5%). Patients with homozygous deletion of interferons exhibited significantly shortened overall or disease-free survival time in a number of cancer types, whereas patients with homozygous deletion of defensins did not significantly associate with

worse overall or disease-free survival. Gene expression analyses suggested that homozygous deletion of interferon and defensin genes could activate genes involved in oncogenic and cell-cycle pathways but repress other genes involved in immune response pathways, suggesting their roles in promoting tumorigenesis and helping cancer cells evade immune surveillance. Further analysis of the whole exomes of 109 patients with melanoma demonstrated that the homozygous deletion of interferon ($P = 0.0029$, OR = 11.8) and defensin ($P = 0.06$, OR = 2.79) genes are significantly associated with resistance to anti-CTLA4 immunotherapy.

Conclusions: Our analysis reveals that the homozygous deletion of interferon and defensin genes is prevalent in human cancers, and importantly this feature can be used as a novel prognostic biomarker for immunotherapy resistance. *Clin Cancer Res*; 24(14): 3299–308. ©2018 AACR.

Introduction

Somatic copy-number alteration (SCNA) is one of the major sources of genomic instabilities that play critical roles in cancer development and progression. Previous studies estimate that 25% of the cancer genome is affected by arm-level SCNAs and 10% by focal SCNAs with 2% overlap (1). Mapping the focal SCNAs recurrent in different cancer types could potentially reveal the common molecular mechanisms of tumorigenesis and identify therapeutic targets. Through analyzing the copy-number profiles of 4,934 tumor genomes across 11 cancer types, The Cancer Genome Atlas (TCGA) project identified 140 recurrent focal SCNAs including 70 amplified regions affecting 959 genes and 70 deleted regions affecting 2,084 genes (2).

Another study exploring the copy-number profiles of 3,131 tumor genomes across 26 cancer types identified 158 recurrent focal SCNAs including 76 amplification affecting 1,566 genes and 82 deletions affecting 2,001 genes (1). However, distilling this genomic alteration information to enable cancer precise medicine is still limited.

Compared with genes with copy-neutral, single-copy loss, copy-gain or amplification, homozygously deleted genes provide a valuable resource to identify genes essential for cancer development, proliferation, and survival. Although similar work has been done in cell lines (3), genome-wide screening for homozygously deleted genes in many different cancer types with a large number of patient specimens representing the heterogeneity of tumors has not been done. In this study, we reanalyzed the copy-number profiles of 10,759 patients across 31 cancer types with the aim to identify recurrent homozygously deleted genes implicated in carcinogenesis. Strikingly, we found 97% of identified homozygously deleted genes are located in only two loci including 9p21.3 and 8p23.3–21. In particular, we found type-I interferon (IFN) gene cluster located in 9p21.3 and defensin (DEF) gene cluster located in 8p23.3–21.1 are homozygously deleted in at least 7% of patients in each of the 19 cancer types. Survival analyses of different tumor types indicated that patients with homozygous deletion of interferon or defensin genes exhibit much worse overall survival (OS) or disease-free survival (DFS). RNA-seq gene expression analyses between patients with and without IFN/DEF deletion in 19 cancer types indicate that homozygous

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Translational Relevance

Immunotherapy has become a major treatment option for many cancers; however, therapeutic resistance is frequently observed, and the genetic basis of lack of response is mostly unknown. By analyzing the genomes of 10,759 patients with cancer across 31 cancer types, we found interferon and defensin genes are homozygously deleted with high frequencies in 19 cancer types, and the surviving time of patients with these deletions is significantly reduced. Further analysis of the genomes of 109 patients with melanoma treated with ipilimumab—a monoclonal antibody targeting CTLA4 demonstrated that the homozygous deletion of type-I interferon and defensin genes are significantly associated with resistance to immunotherapy. Given that interferon and defensin play important roles in tumor immunity, our study revealed a generic mechanism that might be utilized by tumor cells to escape immune destruction and identified that homozygous deletion of interferon/defensin as potential prognostic and predictive biomarker for immunotherapy resistance.

deletions of IFN and DEF activate oncogenic and cell-cycle pathways but repress immune response pathways. Reanalysis of the whole-exome sequencing (WES) data generated from 109 patients with metastatic melanoma suggests that homozygous deletion of IFN and DEF genes are significantly associated with resistance to anti-CTLA4 therapy (4). As a large body of evidence suggests that interferons and defensins play major roles in tumor immunity by recognizing tumor cells and serve as a bridge to spontaneous adaptive T-cell response (5–11), our findings suggest a common molecular mechanism mediated by the deletion of interferon and defensin genes, through which tumor cells escape immune detection and destruction. Given the pervasiveness of homozygous deletion of IFN and DEF genes in primary cancers, this study reveals universal and novel prognostic biomarkers to predict immunotherapy resistance, which has implications for personalized medicine.

Materials and Methods

TCGA copy-number variation data and analysis

The thresholded SCNA data of 10,843 patients (33 cancer types) were downloaded from the Cancer Genome Hub at the University of California at Santa Cruz Biotechnology (<https://genome-cancer.ucsc.edu/>; ref. 12). After removing CHOL ($N = 36$) and DLBC ($N = 48$) cohorts that have less than 50 patients, SCNA profiles of 10,759 patients were analyzed. These 10,759 patients compose 31 cancer types, which include 22 common cancers and nine rare cancers. Common and rare tumor designation is defined by <http://cancergenome.nih.gov/cancersselected/RareTumorCharacterizationProjects>. Matched OS or DFS data were downloaded from CBioPortal (<http://www.cbioportal.org/>; ref. 13). In brief, Gistic2 generated gene-level CNV estimates were thresholded into discrete values $-2, -1, 0, 1, 2$ representing homozygous deletion, single-copy deletion, diploid neutral, low-copy number gain and high-copy number amplification, respectively. Homozygous deletion frequency is

calculated as:

$$\text{Homozygous deletion frequency} = \frac{\{\text{patients with "-2" status}\}}{\{\text{total patients}\}}$$

Gene expression analysis

For each tumor type, patients with cancer were first divided into two groups according to the HDI (homozygous deletion of interferon) and/or HDD (homozygous deletion of defensin) status. Patients without RNA-seq data were removed. Gene-level expression estimates (i.e., RSEM normalized read count) were downloaded from TCGA Data Portal (<https://tcga-data.nci.nih.gov/>). EdgeR was used to compare gene expression profiles between patients with HDI/HDD and those without HDI/HDD. Differentially expressed genes were determined by the FDR cutoff 0.01 (14).

Pathway and IPA analysis

Functional annotation of the homozygously deleted genes was performed by DAVID (<https://david.ncifcrf.gov/>; ref. 15) and ConsensusPathDB gene set overrepresentation analysis (<http://cpdb.molgen.mpg.de/CPDB>; ref. 16). Commonly upregulated genes were defined as genes that significantly upregulated in at least three cancer types but not downregulated in any of the 17 cancer types. Commonly downregulated genes were defined as genes that significantly downregulated in at least three cancer types but not upregulated in any of the 17 cancer types. Commonly upregulated and downregulated genes were overlapped with hallmark gene sets collected in the Molecular Signature Database (<http://software.broadinstitute.org/gsea/msigdb/>). Significant overlaps were determined by FDR q value ≤ 0.05 . Upregulated and downregulated genes in each cancer types were uploaded into <https://apps.ingenuity.com> to do Ingenuity Pathway Analysis (IPA).

Power analysis

We sought to determine if there is sufficient number of events available for OS and DFS analyses for each cancer type. At given statistical significance criterion ($\alpha = 0.05$, two-tailed) and statistical power 80% (i.e., $\beta = 0.2$), the minimum number of required events (K) is calculated as:

$$K = \text{Round} \left(\frac{(Z_{\alpha/2} + Z_{\beta})^2}{\pi_1 \cdot \pi_2 \cdot (\log HR)^2} \right)$$

where $Z_{\alpha/2} = 1.96$, $Z_{\beta} = 0.84$, and π_1 and π_2 are the proportions to be allocated into two groups and were determined by the HDI/HDD frequencies in each cancer type. HR is the hazard ratio estimated using the log-rank approach implemented in the survival package (<https://cran.r-project.org/web/packages/survival/index.html>).

$$HR = \frac{O_a/E_a}{O_b/E_b}$$

where O_a and E_a are the numbers of observed and expected events in patients with HDI/HDD, and O_b and E_b are the numbers of observed and expected events in patients without HDI/HDD. $HR > 1$ indicates higher hazard of death or progression from the HDI/HDD group.

Survival and statistical analysis

To increase sample size, we combined COAD and READ as colorectal cancer and LUAD and LUSC as lung cancer (therefore

31 cancer types reduced to 29). We removed four rare tumors having no OS and DFS time available for survival analysis including adrenocortical carcinoma (ACC), mesothelioma (MESO), pheochromocytoma, and paraganglioma (PCPG) and uterine carcinosarcoma (UCS). According to the power analysis described above, all cancer types except LGG were underpowered; we, therefore, required at least 10 events (i.e., death or progression) in the HDI/HDD group. Based on this criterion, we further removed 10 cancer types having less than 10 events for both OS and DFS in the HDI/HDD group including cervical squamous cell carcinoma and endocervical adenocarcinoma (CESC), acute myeloid leukemia (LAML), kidney chromophobe (KICH, rare tumor), kidney renal clear cell carcinoma (KIRC), kidney renal papillary cell carcinoma (KIRP), testicular germ cell tumors (TGCTs, rare tumor), thyroid carcinoma (THCA), thymoma (THYM, rare tumor), uterine corpus endometrial carcinoma (UCEC), and uveal melanoma (UVM, rare tumor). Using the same criterion, PRAD (OS, $N_{\text{events}} = 2$), ESCA (OS, $N_{\text{events}} = 6$), and SARC (DFS, $N_{\text{events}} = 8$) were also removed. We performed either OS or DFS analyses for the remaining 15 cancer types.

Survival analysis was performed using the "survival" R package available from <http://cran.r-project.org>. The log-rank test was used to evaluate if the difference in OS or DFS between the above two subgroups was statistically significant. Benjamini-Hochberg (BH) method was used to adjust log-rank test P values for multiple comparisons. CoMET exact test was used to evaluate the mutual exclusivity of HDIs and HDDs in each tumor type (17). Fisher exact test was used to evaluate the significance of association between HDI/HDD events and ipilimumab treatment responses in patients with melanoma. Wilcoxon rank-sum test (a.k.a. Mann-Whitney U test) was used to evaluate the significance of the difference in diagnosis ages between patients with LUSC with concurrent HDI/HDD and those without. Wilcoxon rank-sum test was also used to compare HDI/HDD frequencies between common and rare cancer types.

Melanoma whole-exome sequencing analysis

The whole-exome sequencing data of tumors and matched normal tissues (i.e., adjacent normal tissues or peripheral blood mononuclear cells) for 109 patients with melanoma were downloaded from dbGAP with accession number phs000452.v2.p1 (4). One patient (Pat121) was not included in the analysis because the file is larger than the maximum size allowed to download. Treatment responses to anti-CTLA4 were retrieved from the supplementary data of the original publication. Raw reads alignments to the human reference genome (hg19/GRCh37) were extracted from the original SRA files using SRA-Toolkit (18). Somatic copy-number variation (i.e., copy-number change in tumor tissue vs. normal tissues) for each patient was called by VarScan-2 with the default parameters (19). The postprocessing step of smoothing, segmentation, and re-center were performed by the DNACopy R package and in-house scripts (20). Genes with \log_2 (tumor/normal) smaller than -1 were considered as homozygous deletions. Patients were stratified into "responder" ($N = 27$), "nonresponder" ($N = 72$), and "long-term survivor" ($N = 10$) following the original publication (4). Specifically, "responders" referred to patients who achieved clinical benefits ["complete response" or "partial response" or "stable disease" with OS > 1 year by RECIST criteria]. "Nonresponder" referred to those with minimal or no benefit from ipilimumab ("progressive disease"

or "stable disease" with OS < 1 year by RECIST criteria). "Long-term survivors" refer to a cohort of patients who achieved long-term survival (OS > 2 years) after ipilimumab treatment but with early tumor progression [i.e., shorter progression-free survival (PFS) < 6 months].

Results

Homozygous deletions of interferon and defensin genes are pervasive in human cancers

To identify the common, homozygously deleted genes in human cancers, we analyzed the copy-number profiles of 10,759 patients across 31 cancer types (Supplementary Table S1). We identified 242 genes whose average homozygous deletion frequencies across 31 cancer types are higher than 3% (Supplementary Table S2). Strikingly, we found 235/242 (97%) genes are clustered on either 9p21.3 or 8p23.3–21.1 with only seven (3%) genes (*PTEN*, *RB1*, *DMD*, *PDE4D*, *WWOX*, *LRP1B*, and *CCSER1*) residing on other chromosomes (Fig. 1A; Supplementary Table S2). As expected, all these seven genes except *CCSER1* (alias *FAM190A*) are known tumor suppressors and have been identified as the potential drivers of focal deletions detected by previous studies (1, 2). The molecular function *CCSER1* is largely unknown, but its homozygous deletion has also been observed in various cancers (21–23). Functional classification of the 242 genes revealed that interferons (16 genes, q value = 8.37×10^{-20}) and defensins (24 genes, q value = 1.65×10^{-30}) are the most significantly enriched terms (Supplementary Table S3). The 16 type-I interferon genes are located on 9p21.3, which include 13 IFN α genes, 1 IFN β , 1 IFN ϵ , and 1 IFN ω gene (Supplementary Fig. S1A). The 24 defensin genes are located on 8p23.3–21.1, which include 6 α -defensin and 18 β -defensin genes (Supplementary Fig. S1B). As both interferons and defensins are involved in innate immune response and play important roles in recognizing tumor cells and inducing an antitumor immune response, the widespread, homozygous deletion of these genes suggests a common molecular mechanism through which tumor cells escape immune destruction.

We observed the homozygous deletion of interferons (HDIs) and defensins (HDDs) in all 31 cancer types except PCPG (pheochromocytoma and paraganglioma, $N = 162$), THCA (thyroid carcinoma, $N = 501$), and LAML (acute myeloid leukemia, $N = 191$; Fig. 1B; Supplementary Table S4). Interestingly, we found the frequencies of HDI/HDD are much higher in the 22 common cancer types (median frequency = 10%) than those in the nine rare cancer types (median frequency = 3%; $P = 0.1$, two-tailed Wilcoxon rank-sum test; Supplementary Fig. S2). This result suggests HDI/HDD as a common molecular mechanism involved primarily in the carcinogenesis of common cancers. Using 5% alternation frequency as the threshold, we defined 19 cancer types as the high HDI/HDD group, in which two brain tumor types LGG (brain lower-grade glioma) and glioblastoma multiforme (GBM) exhibit the lowest (7%) and highest (31%) alternation frequencies, respectively (Fig. 1B; Supplementary Table S4). We further divided the 19 cancer types into three subtypes using the prevalence ratio ($PR = \{\# \text{ of HDIs}\} / \{\# \text{ of HDDs}\}$) of 5 as the threshold: The I type is defined as cancer types with HDI at least five times more ($PR \geq 5$) prevalent than HDD, including LGG, GBM, PAAD (pancreatic adenocarcinoma), and MESO (mesothelioma; Fig. 2A and B; Supplementary Fig. S3A and S3B). The D type is defined as cancer types with HDD at least

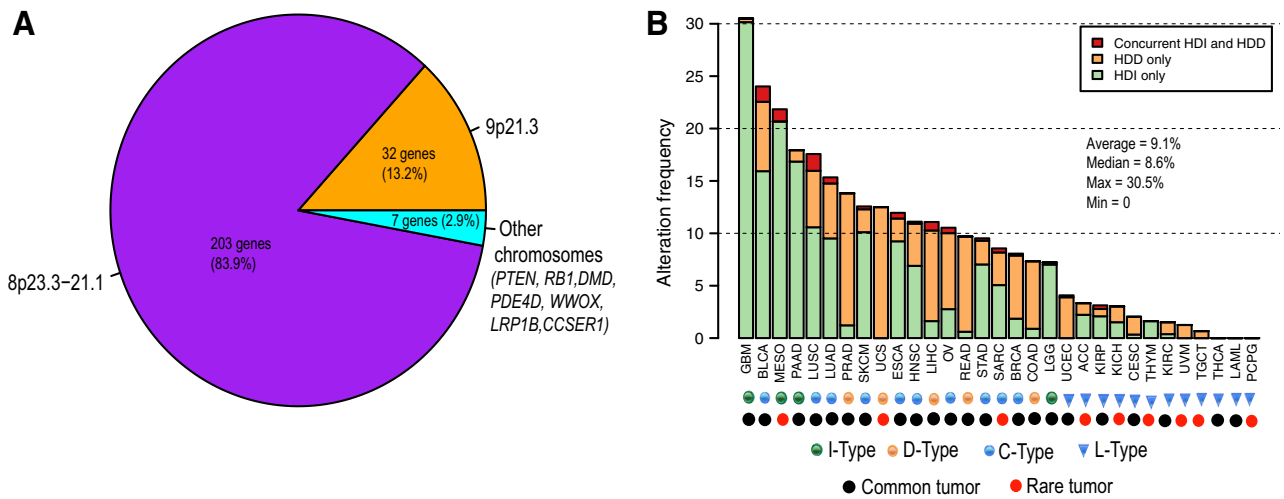


Figure 1.

Genomic distribution of homozygously deleted genes and the deletion frequency of HDI/HDD in each tumor type. **A**, Genomic distribution of the 242 homozygously deleted genes identified. **B**, Alteration frequency of HDIs and HDDs in 31 cancer types. Patients of each cancer type were divided into three groups: patients with HDI only (green), patients with HDD only (orange), and patients with concurrent HDI and HDD (red). The 31 cancers were classified into four types: L type refers to cancer types with HDI/HDD frequencies less than 5% (blue triangle); I type refers to cancer types in which HDI is the dominant alteration (green circle); D type refers to cancer types in which HDD is the dominant alteration (orange circle); C type refers to cancer types in which both HDI and HDD are prevalent (blue circle). Common and rare tumors are designated by TCGA and indicated using black and red circles, respectively. HDI, homozygous deletion of type-I interferons; HDD, homozygous deletion of defensin.

five times more ($PR \leq 0.2$) prevalent than those of HDI, including prostate adenocarcinoma (PRAD), colon adenocarcinoma (COAD), rectum adenocarcinoma (READ), liver hepatocellular carcinoma (LIHC), uterine carcinosarcoma (UCS; Fig. 2C and D; Supplementary Fig. S3C and S3D). The C type refers to those cancer types with both HDI and HDD ($0.2 < PR < 5$), including bladder urothelial carcinoma (BLCA), lung adenocarcinoma (LUAD), lung squamous cell carcinoma (LUSC), breast invasive carcinoma (BRCA), ovarian serous cystadenocarcinoma (OV), head and neck squamous cell carcinoma (HNSC), skin cutaneous melanoma (SKCM), esophageal carcinoma (ESCA), sarcoma (SARC), and stomach adenocarcinoma (STAD; Fig. 2E and F; Supplementary Fig. S3E–S3H).

As shown in Fig. 2 and Supplementary Fig. S3, I-type tumors almost exclusively have HDI but not HDD, and D-type tumors almost exclusively have HDDs but not HDIs. Even for C-type tumor such as BRCA ($N = 1,079$), there were 20 (2%) patients having HDIs and 65 (6%) patients having HDDs but only two (0.2%) patients with concurrent HDI and HDD. These data suggested that HDI and HDD had the tendency toward mutually exclusive both within and across cancer types, even though they did not reach statistical significance due to the limited number of cases (Supplementary Table S4). We expect patients with concurrent HDI and HDD would have a worse prognosis than those patients with only HDI or HDD, but the paucity of concurrent HDI and HDD preclude rigorous statistical analysis (Supplementary Table S4). However, we found in the LUSC cohort which has the most concurrent HDI and HDD cases ($N = 8$), the diagnosis age of patients with concurrent HDI and HDD (median = 60) is much smaller than other patients (median = 68; $P = 0.079$, two-tailed Wilcoxon rank-sum test; Supplementary Fig. S4). This result suggests the association between concurrent HDI and HDD and the early tumor onset in LUSC.

Homozygous deletions of interferon or defensin genes associate with worse OS or DFS in human cancers

As both interferons and defensins are implicated in antitumor immunity, we next investigated if patients with HDI or HDD lesions exhibit worse OS or DFS. Indeed, we observed patients with I-type tumors (Fig. 3A and B) D-type tumors (Fig. 3C and D), and C-type tumors (Fig. 3E and F) had significantly worse OS or DFS. For examples, the median OS time of patients with LGG with HDI or HDD (24 months) is only 26% of those patients without HDI or HDD (93 months; $P = 2.02 \times 10^{-10}$, log-rank test; Fig. 3A). Similarly, the median DFS time of patients with bladder cancer with HDI or HDD (17.5 months) is only 40% of those patients without HDI or HDD (44 months); $P = 0.0026$; Fig. 3E), and the median OS time of patients with lung cancer with HDI or HDD is 70% of those patients without HDI or HDD ($P = 0.0085$; Fig. 3F). We found that, in most cancer types, patients with HDI/HDD lesions consistently exhibit worse OS or DFS compared with those without HDI/HDD lesions, even though some cancer types did not reach statistical significance (Supplementary Fig. S5). The associations of HDI/HDD status with worse clinical outcomes across cancer types highlight their prognostic values.

We found that IFN genes are located approximately 485 kb to 890 kb away from a well-known tumor suppressor *CDKN2A* (cyclin-dependent kinase inhibitor 2A; Supplementary Fig. S1A). The homozygous deletion of these genes has been reported in mesothelioma and numerous cancer cell lines (3, 24, 25). We asked the question whether homozygous deletions of interferon genes are passive hitchhiking events due to the nearby *CDKN2A* deletion, or they play an active role in tumorigenesis and affect the patient survival. Therefore, we tested if the additional loss of IFN genes could further reduce patients' OS or DFS, comparing with patients with *CDKN2A* deletion only. We performed the survival

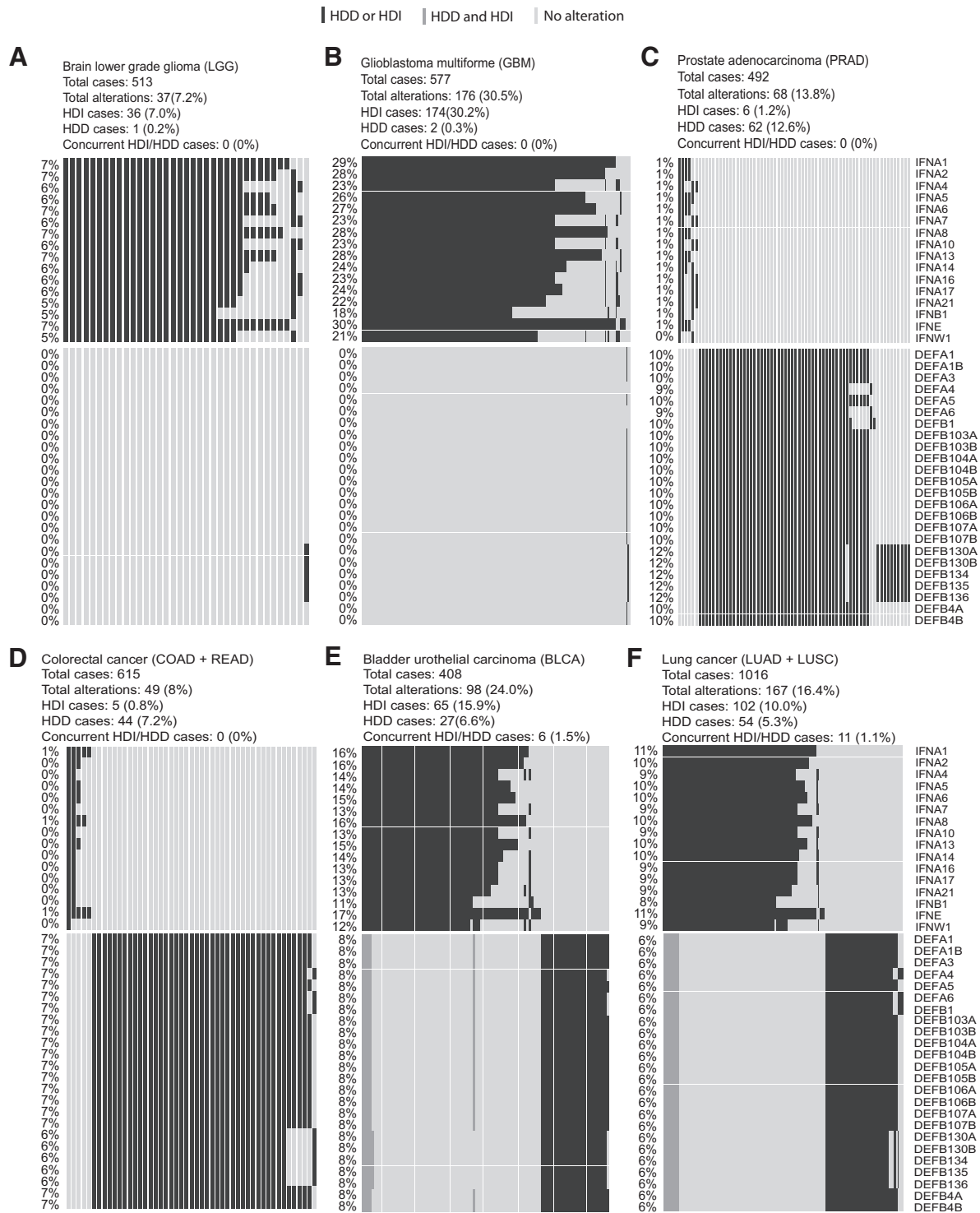
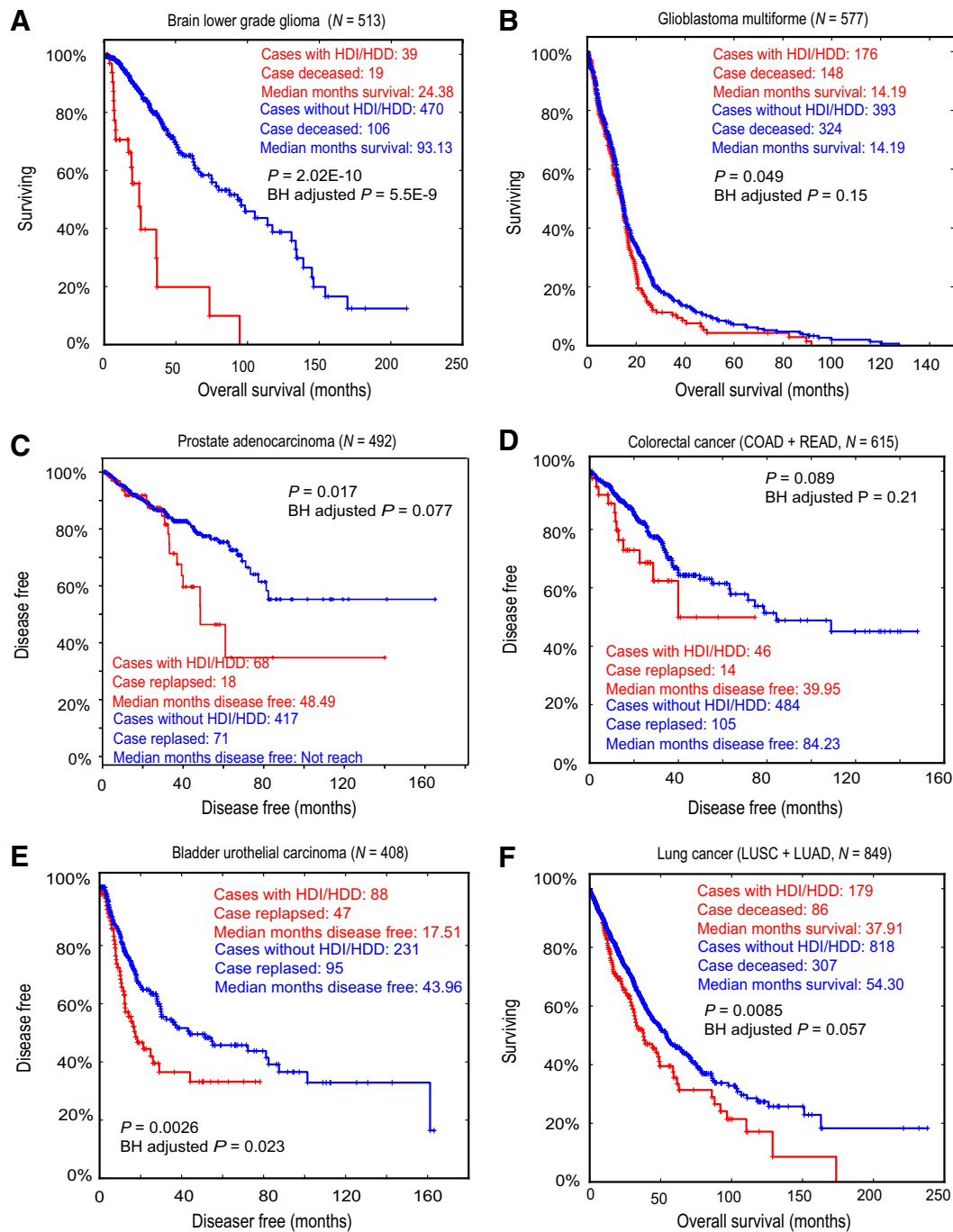


Figure 2. Homozygous deletions of interferon and defensin genes in six cancer types. In each panel, rows represent genes and columns represent patients. Black bars, patients with HDD or HDI, gray bars, patients with concurrent HDD and HDI. Overall deletion frequency of each gene is listed on the left. **A** and **B**, I-type cancers using brain lower-grade glioma (LGG) and GBM as examples. **C** and **D**, D-type cancers using PRAD and colorectal cancer as examples. Colorectal cancer is the combined cohort of colon adenocarcinoma (COAD) and rectum adenocarcinoma (READ). **E** and **F**, C-type cancers using BLCA and lung cancer as examples. Lung cancer is the combined cohort of lung adenocarcinoma (LUAD) and lung squamous cell carcinoma (LUSC). HDI, homozygous deletion of type-I interferons; HDD, homozygous deletion of defensin.

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**Figure 3.**

Comparing the OS or DFS between patients with (red) and without (blue) HDIs/HDDs. **A**, OS curves for brain lower-grade glioma (LGG). **B**, OS curves for GBM. **C**, DFS curves for PRAD. **D**, DFS curves for colorectal cancer. Colorectal cancer is the combined cohort of colon adenocarcinoma (COAD) and rectum adenocarcinoma (READ). **E**, DFS curves for BLCA. **F**, OS curves for lung cancers. Lung cancer is the combined cohort of lung adenocarcinoma (LUAD) and lung squamous cell carcinoma (LUSC).

analyses on three patient groups: (i) patients only have *CDKN2A* deletion ($CDKN2A^{-}/IFN^{+}$); (ii) patients have *CDKN2A* deletion and additional *IFN* gene deletions ($CDKN2A^{-}/IFN^{-}$); and (iii) patients have neither *CDKN2A* nor *IFN* gene deletions ($CDKN2A^{+}/IFN^{+}$). Very few patients have *IFN* deletion only

(i.e., $CDKN2A^{+}/IFN^{-}$), so this group was excluded from our analysis. As expected, $CDKN2A^{-}/IFN^{-}$ patients have the worst prognosis compared with $CDKN2A^{-}/IFN^{+}$ and $CDKN2A^{+}/IFN^{+}$ groups in a variety of cancer types, including LGG, GBM, BRCA, lung cancer (LUAD + LUSC), colorectal cancer (COAD + READ),

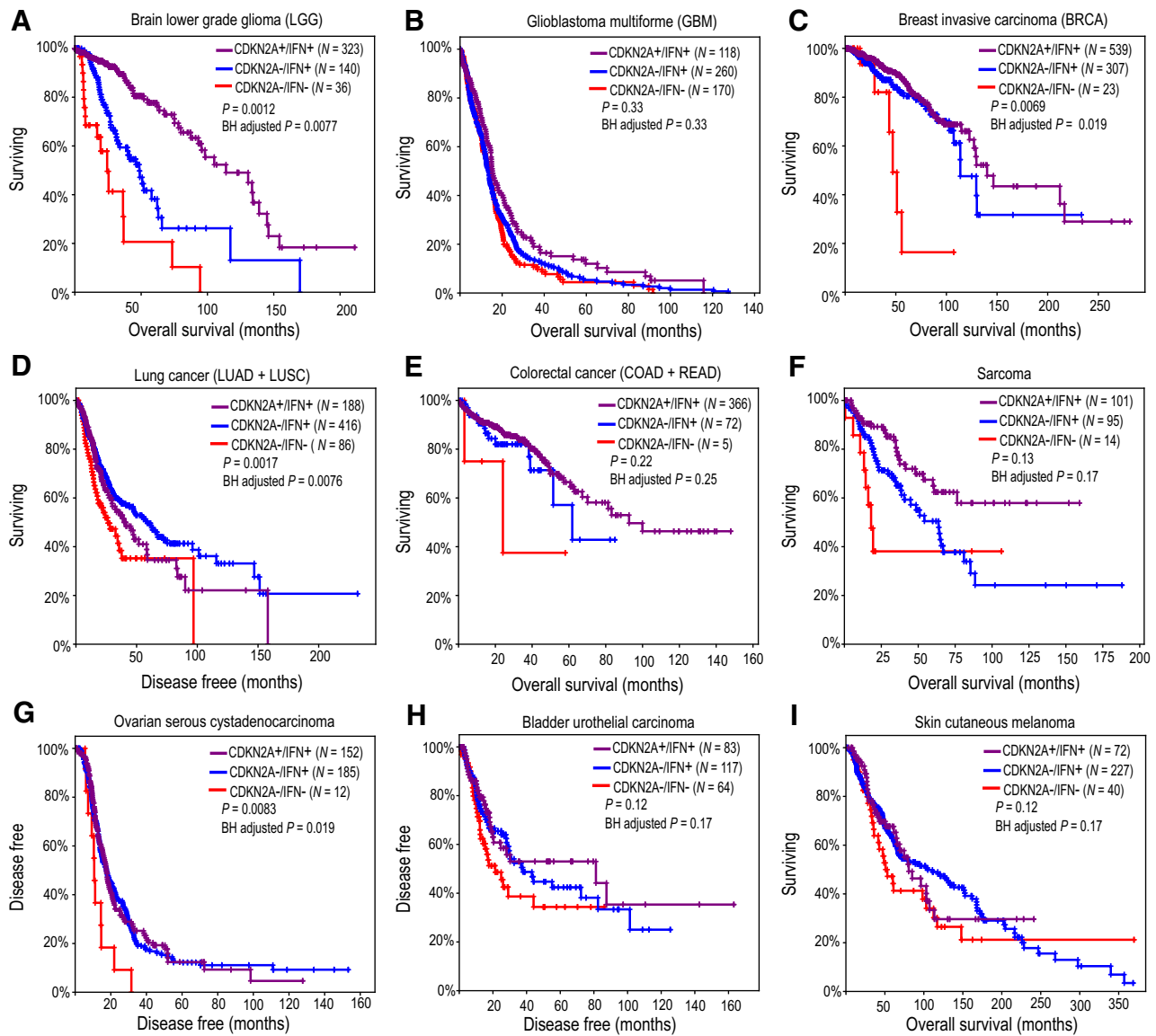


Figure 4. Comparing the OS between patients having neither *CDKN2A* nor interferon gene deletions (purple), patients having *CDKN2A* deletion only (blue), and patients having both *CDKN2A* and interferon gene deletions (red). Survival curves for brain lower-grade glioma (LGG), GBM, breast invasive carcinoma (BRCA), lung cancer, colorectal cancer, sarcoma (SARC), ovarian serous cystadenocarcinoma (OV), BLCA, skin cutaneous melanoma (SKCM) are indicated in **A** to **I**, respectively. Colorectal cancer is the combined cohort of colon adenocarcinoma (COAD) and rectum adenocarcinoma (READ). Lung cancer is the combined cohort of lung adenocarcinoma (LUAD) and lung squamous cell carcinoma (LUSC). To increase sample size, patients with single-copy loss of *CDKN2A*, truncated mutations of *CDKN2A*/*IFNs*/*DEFs* were also included. Log-rank test *P* values were calculated by comparing patients having *CDKN2A* deletion only (blue) with patients having both *CDKN2A* and interferon gene deletions (red).

SARC, OV, BLCA, SKCM, ESCA, and PAAD (Fig. 4; Supplementary Fig. S6). After performing power analysis (see Materials and Methods), we found all tumor types except LGG are underpowered (Supplementary Table S5). Therefore, the insignificant *P* values of OS/DFS analyses in some tumor types are most likely due to limited sample size. For instance, only five and 14 patients had both *CDKN2A* and *IFN* gene deletions for colorectal cancer and SARC, respectively. It could be also due to other confounding factors such as *PTEN* and *RB1* deletions, which tend to be mutually exclusive with HDIs and HDDs (Supplementary Fig. S7).

Homozygous deletions of interferon and defensin genes activate oncogenic and cell-cycle pathways but repress immune response pathways

To identify gene expression signatures associated with the deletion of *IFN* and *DEF* genes, we performed gene expression analysis on each of the 19 cancer types (or 17 cancer types when COAD and READ are combined as colorectal cancer, LUSC and LUAD are combined as lung cancer) having high frequencies of HDI and HDD. Through differential gene expression analyses between patients with and without HDI/HDD lesions in 17 cancer

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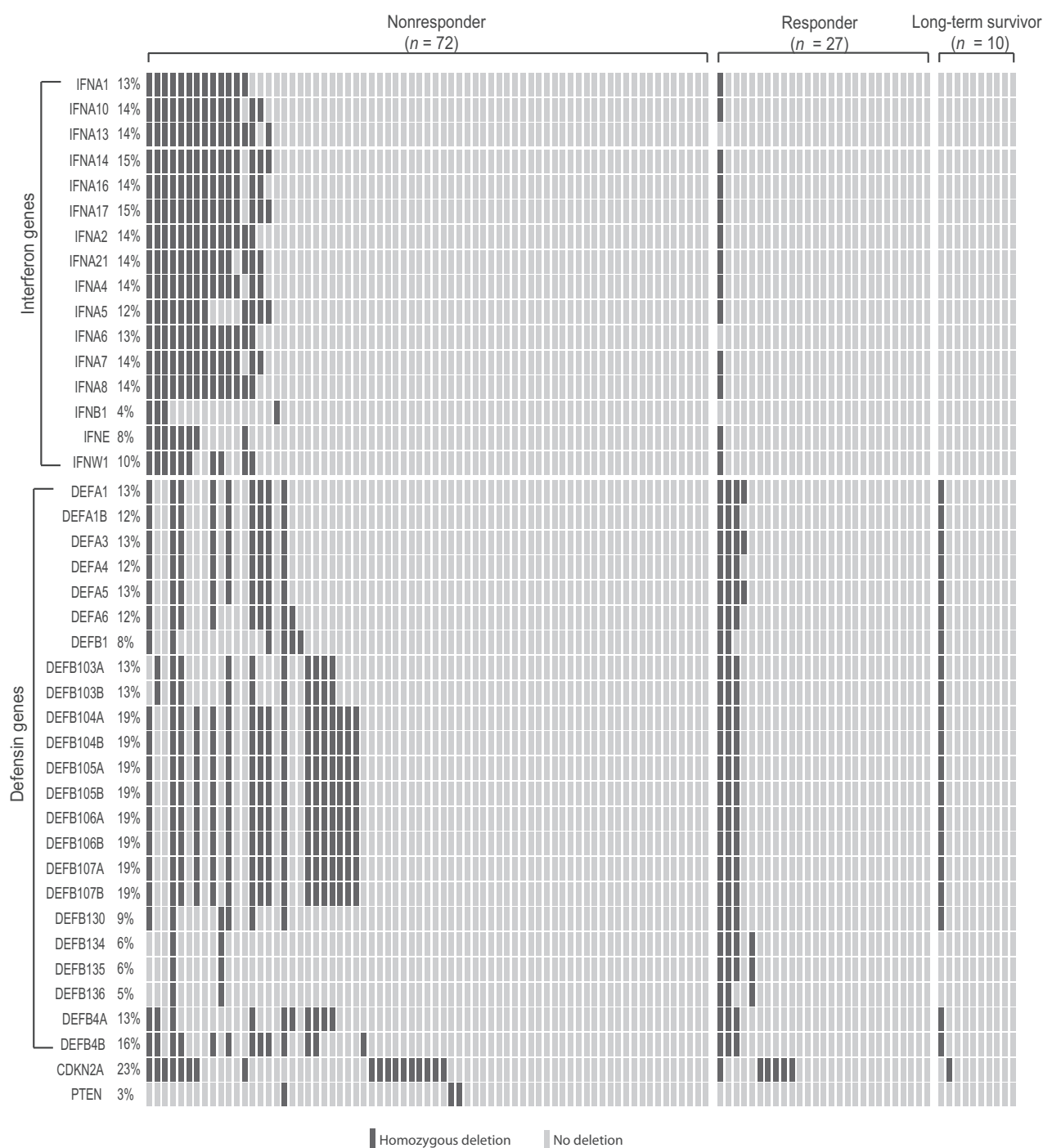


Figure 5. HDI/HDD alteration frequencies in 109 patients with melanoma treated with ipilimumab. Oncoprint plot showing the deletion landscape of interferon genes, defensin genes, *CDKN2A* and *PTEN* in 109 patients with metastatic melanoma. Patients were divided into three groups including nonresponder ($N = 72$), responder ($N = 27$), and long-term survival ($N = 10$), according to the response outcomes to ipilimumab. HDI, homozygous deletion of type-I interferons; HDD, homozygous deletion of defensin.

types, we detected a total of 4,599 genes that differentially expressed in at least one cancer type (Supplementary Table S6; Supplementary Fig. S8A). On average, 204 (median = 145) and 254 (median = 232) genes are significantly ($FDR < 0.01$) upre-

gulated and downregulated in each cancer type, respectively (Supplementary Fig. S8B).

To identify the implicated molecular pathway, we defined a set of genes commonly altered in multiple cancer types (see Materials

and Methods) and overlapped them with the hallmark gene sets collected in Molecular Signature Database (<http://software.broadinstitute.org/gsea/msigdb/index.jsp>). We found "genes defining epithelial–mesenchymal transition (EMT)" ($FDR = 4.6 \times 10^{-18}$), "genes upregulated by KRAS activation" ($FDR = 6.7 \times 10^{-9}$), and cell-cycle genes ($FDR = 1.3 \times 10^{-6}$) are significantly enriched in genes upregulated in patients with HDI/HDD (Supplementary Table S7). EMT is a biologic process involved in cancer progression and metastasis (26). *KRAS* is a proto-oncogene and its activation is implicated in various malignancies. Similarly, "genes regulated by NF- κ B in response to tumor necrosis factor (TNF)" ($FDR = 1.9 \times 10^{-11}$), "genes upregulated by STAT5 in response to IL2 stimulation" ($FDR = 3.0 \times 10^{-8}$) are significantly enriched in genes downregulated in patients with HDI/HDD (Supplementary Table S7). It is known that NF- κ B family members play key roles in regulating the transcription of cytokines and antimicrobial effectors and thereby controlling innate and adaptive immune responses (27) and that IL2 has essential roles in regulating immune tolerance through its direct effects on T cells (28).

We further performed IPA analyses to identify common upstream regulators for genes differentially expressed between the two groups for each tumor type. Strikingly, we found that TNF is detected as the top upstream regulator for all the 17 cancer types with extremely significant *P* values (Supplementary Table S8). IFN γ (IFNG) is detected as the second most common upstream regulator in 10 cancer types including MESO, PAAD, LGG, COAD, READ, LUSC, LUAD, BLCA, SARC, and STAD (Supplementary Table S8). Both TNF and IFN γ have been found to upregulate immune checkpoint molecule PD-L1 (B7-H1) in a broad range of tumors (29) and have been linked to resistance to T-cell therapy and anti-CTLA4 therapy in melanomas (30–32). In conclusion, HDD and HDI activate genes involved in oncogenic/cell-cycle pathways and repress genes involved in immune response demonstrating their active role in facilitating tumorigenesis and immune escape.

Homozygous deletions of interferon/defensin genes associate with immunotherapy resistance in melanomas

In a recent report, copy loss of type-I interferon genes is found in six out of 12 patients with melanoma that resist to anti-CTLA4 treatment, but in none of the four responders ($P = 0.23$, two-tailed Fisher exact test; 31). To test if HDI and HDD are associated with immunotherapy resistance in an independent larger cohort, we analyzed the whole-exome sequencing data of 109 patients with metastatic melanoma treated with ipilimumab—a monoclonal antibody targeting CTLA4 (4). The 109 patients with melanoma are stratified into 72 nonresponders (i.e., patients resistant to ipilimumab treatment), 27 responders, and 10 long-term survivors according to RECIST criteria (see Materials and Methods; ref. 33). HDIs are detected in 25% (18/72) of nonresponders, 4%

Table 2. Contingency table evaluating the association between HDD and nonresponsiveness to ipilimumab treatment

	α -, β -DEFs (homozygous deletion)	α -, β -DEFs (1 or more copies)	Total
Nonresponder	22	50	72
Responder + long-term survivor	5	32	37
Total	27	82	109

NOTE: Two-tailed Fisher exact test, $P = 0.06$, odds ratio = 2.79. Abbreviation: HDD, homozygous deletion of defensin.

(1/27) responders, and none (0/10) of the long-term survivors (Fig. 5). Association analysis suggested that HDI is significantly associated with nonresponders ($P = 0.0029$, odds ratio = 11.8; Table 1). HDDs are also detected more frequently in nonresponders, and the association is close to being statistically significant ($P = 0.06$, odds ratio = 2.79; Table 2). As a comparison, deletions of two tumor suppressor genes *CDKN2A* ($P = 0.63$) and *PTEN* ($P = 0.55$) are not associated with responders or nonresponders (Fig. 5). Taken together, our meta-analyses of two independent melanoma cohorts suggest the deletion of interferon and defensin genes is significantly associated anti-CTLA4 treatment resistance and might be a potential prognostic biomarker to predict resistance to other immunotherapies.

Discussion

Immunotherapies have been demonstrated to be efficacious in many cancer types (34–36); however, therapeutic resistance is frequently observed and the mechanisms of both *de novo* and acquired immune resistance are mostly unknown. In this study, we analyzed the copy-number profiles of 10,759 primary cancer tissues and found that the homozygous deletions of type-I interferon and defensin genes are pervasive in 19 TCGA cancer types, and the alteration frequency is much higher than those of well-known tumor suppressors *PTEN* and *RB1* in the same cancer type (Supplementary Fig. S7). More importantly, homozygous deletions of interferon and defensin genes associate with worse OS or DFS, and the resistance to anti-CTLA4 treatment in patients with melanoma. Defects in the type-I interferon signaling pathway have been proposed as a potential mechanism of cancer escape (insensitivity) to immunotherapy in mice and prostate cancer cell line (37, 38). In mice, type-I interferon signals are required to initiate the antitumor CD8⁺ response, and mice without IFN α / β receptor cannot reject immunogenic tumor cells (9, 10). Consistent with these preclinical observations, our observation that type-I interferon genes being frequently deleted in many tumor types suggested a generic mechanism through which tumors develop immunoresistance and revealed new biomarkers to identify responsive patients. Homozygous deletions of interferon and defensin genes activate oncogenic pathways and repress immune response pathways further recapitulate their roles in promoting tumorigenesis and help tumor cells escape immunosurveillance.

Disclosure of Potential Conflicts of Interest

H. Liang is an employee of and has ownership interests (including patents) at and is a consultant/advisory board member for Precision Scientific Ltd. No potential conflicts of interest were disclosed by the other authors.

Table 1. Contingency table evaluating the association between HDIs and nonresponsiveness to ipilimumab treatment

	Type-I IFNs (homozygous deletion)	Type-I IFNs (one or more copies)	Total
Nonresponder	18	54	72
Responder + long-term survivor	1	36	37
Total	19	90	109

NOTE: Two-tailed Fisher exact test, $P = 0.0029$, odds ratio = 11.8. Abbreviation: HDIs, homozygous deletion of type-I interferons.

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