Original Article

Potential predictive value of *JAK2* expression for Pan-cancer response to PD-1 blockade immunotherapy

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Background: Recent clinical studies have shown promise for targeting programmed cell death protein-1 (PD-1) and programmed cell death ligand 1 (PD-L1) signaling in malignant tumors. However, reliable biomarkers for predicting who would benefit from anti-PD-1/PD-L1 inhibitors have not been fully elucidated.

Methods: Here, patients from The Cancer Genome Atlas Pan-Cancer database (N=9,315) were classified into three groups based on the tri-sectional quantiles of their Janus kinase 2 (*JAK2*) RNA expression levels. Sample mRNA expression of PD-L1 and mutational load, CD8A expression [representing CD8+ cytolytic T lymphocytes (CTLs)], cytolytic activity ("CYT") expression, and viral association were compared among groups.

Results: High mRNA expression and gene amplification of PD-L1 were both significantly associated with high *JAK2* expression (P<0.0001). The high *JAK2* expression group exhibited significantly more somatic mutations and neoantigens than did the other groups (P<0.01). CD8A expression, CYT, and oncogenic virus infection were each notably associated with high *JAK2* expression (P<0.0001).

Conclusions: In conclusion, high *JAK2* expression was associated with high mRNA expression of PD-L1, CD8+CTLs and mutational burdens, CYT expression, and oncogenic viral infection. This comprehensive analysis demonstrated the important value of assessing *JAK2* expression to predict responders to immunotherapy.

Keywords: Immunotherapy; Janus kinase 2 (JAK2); programmed cell death protein1 (PD-1); The Cancer Genome Atlas (TCGA)

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Introduction

Immunotherapy represents a recent major breakthrough in cancer treatment. In particular, programmed cell death protein-1 (PD-1), programmed cell death ligand 1 (PD-L1), and PD-L2 pathways constitute key immune checkpoints. The PD-1 inhibitors nivolumab and pembrolizumab have induced durable control and shown a survival benefit in immunogenic tumors, such as non-small cell lung carcinoma, melanoma, renal cell carcinoma, and head and neck cancer (1).

Persisting expression of PD-L1 on the surface of tumor cells and partial immune cells can be induced by tumor cell intrinsic and extrinsic signals, which leads to

immune escape of the tumor (2,3). An increasing number of clinical trials have shown a high objective response in patients with positive PD-L1 expression in tumor samples (4,5). It was also revealed that the number of tumor infiltrating lymphocytes was significantly associated with objective response toward anti-PD-1/PD-L1 therapy (6). Furthermore, recent advances in immuno- genomics have demonstrated that tumors with a status of high mutational burden, abundant neoantigen, and microsatellite-instability-high (MSI-H) demonstrated active response to anti-PD-1/PD-L1 therapy and longer overall patient survival (7-9). Additionally, oncogenic viruses such as Epstein-Barr virus (EBV) or human papillomavirus (HPV) were also associated with an inflamed tumor microenvironment, which potentially resulted in a favorable clinical outcome in response to anti-PD-1/PD-L1 therapy (10,11). Moreover, the cytolytic activity ("CYT"), which was assessed by measuring granzyme A (*GZMA*) and perforin 1 (*PRF1*) expression levels, was associated with inflamed tumors and was considered to be influenced by the infiltration of $CD8^+$ cytolytic T lymphocytes (CTLs) (12,13). Thus, several factors were found to facilitate the antitumor activity of the immune checkpoint inhibitors mentioned above, which raises the question of whether a clear biomarker exists that correlates with these factors.

Notably, current studies have discovered that Janus kinase 2 (JAK2), a classical inflammatory factor, showed a significant correlation with PD-L1, encoded by the *CD274* gene (14). Specifically, a cryptic *JAK2-CD274* rearrangement was generated by a microdeletion spanning the 3'*JAK2*-5'*CD274* region (15). Furthermore, in head and neck cancer, a significant association was found between PD-L1 expression and phosphorylation of JAK2 as detected by immunohistochemistry (16). Patients with melanoma carrying a *JAK2* mutation exhibit an acquired resistance to anti-PD-1/PD-L1 therapy (17); a similar situation has been discovered in a mouse model of breast tumor and melanoma (18). Based on these findings, we speculated that *JAK2* expression might represent a potential biomarker for response to PD-1 blockade immunotherapy.

In the current study, we classified a large set of TCGA Pan-Cancer samples into three groups by measuring their *JAK2* mRNA expression levels. The object of this TCGA Pan-Cancer analysis was to determine the associations between *JAK2* status and the mRNA expression and mutational burden of PD-L1, $CD8^+$CTLs (as measured by *CD8A* expression), CYT expression, and oncogenic viral infection, which would likely provide strategic information for guiding the treatment of immune checkpoint blockade.

Methods

Experimental design

We studied 9,315 samples from The Cancer Genome Atlas (TCGA) database, involving 31 types of cancers. RNA sequencing (RNA-Seq) data of level 3 reads per kilobase of transcript per million mapped reads (RPKM) were obtained from TCGA Data Portal (https://gdc-portal.nci.nih.gov/) and log2-transformed. Amplification of the locus for PD-L1, MSI status, infection of oncogenic viruses, mutation burden, and neoantigen number were analyzed in this study. The capacity of samples varied from different indices owing to data availability. The MSI status was available for 1,010 samples including samples of colon and rectal adenocarcinoma (COAD) (N=285), uterine carcinosarcoma (UCS) (N=55), esophageal carcinoma (ESCA) (N=88), stomach adenocarcinoma (STAD) (N=414), and uterine corpus endometrioid carcinoma (UCEC) (N=168). The infection status of oncogenic viruses, such as EBV, HPV, and hepatitis B virus (HBV) was available in 6,385 samples. Somatic mutational of 6,257 samples and neoantigens of 3,763 were accessible. Altogether, samples of 31 cancer types (N=9,315) were included in the analysis.

Statistical analyses

According to the log 2-transformed RPKM values of *JAK2*, all of the TCGA samples were divided into three groups as follows: High-*JAK2* ($\log_2$*JAK2* ≥8.6037, N=3,105), Medium-*JAK2* (7.8019≤ $\log_2$*JAK2* <8.6037, N=3,105), and Low-*JAK2* ($\log_2$*JAK2* <7.8019, N=3,105). Several predicted biomarkers, such as somatic mutations, neoantigens, *CD8A* expression level, CYT activity, and the mRNA expression of PD-L1, were also log2- transformed. The statistical correlations between variables including the above biomarkers, *JAK2*, and oncogenic viruses were analyzed. The association between MSI status and *JAK2* expression was tested in all samples. Statistical methods including the Mann-Whitney U and correlation analysis were applied in genomic data analysis. Statistical analyses were conducted using GraphPad Prism (version 7.0, LaJolla, CA) Scatter dot plot and box and whisker plots indicate median and 95% confidence intervals (CI), and Chi-square values. All reported P values were two-tailed and for all analyses, P≤0.05 was considered statistically significant, unless otherwise specified.

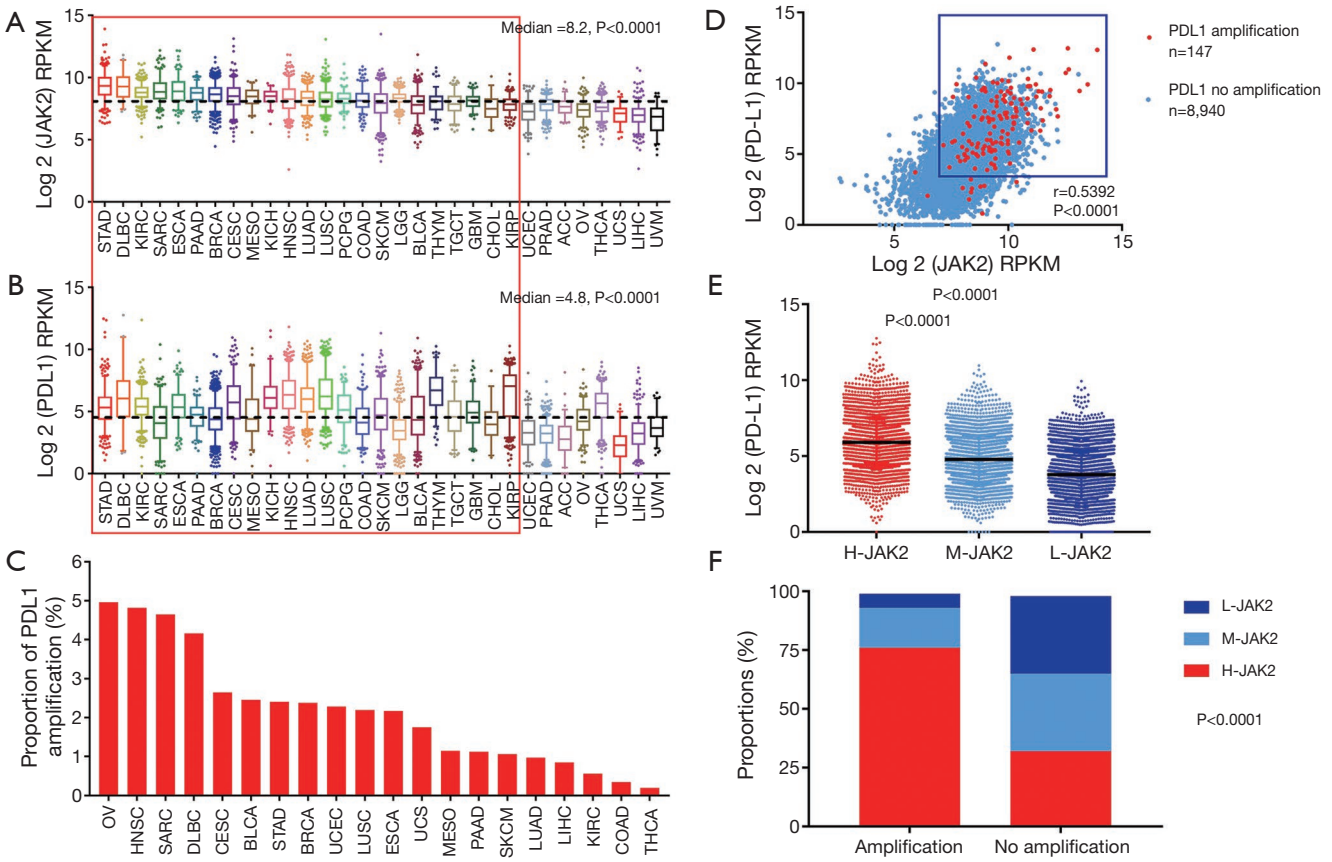

Figure 1 High Janus kinase 2 (*JAK2*) expression is associated with mRNA expression and amplification of the gene for programmed cell death ligand 1 (PD-L1). (A,B) The distribution of mRNA expression for JAK2 and PD-L1 in thirty-one cancer types is shown according to analysis of values from The Cancer Genome Atlas (TCGA) database. The mRNA expression values for JAK2 and PD-L1 are log2-transformed. (C) Proportion of gene locus amplification for *PD-L1* in these cancer types is shown. (D) Correlation between mRNA expression of JAK2 and PD-L1 according the cancer types in TCGA. The association between amplification of the gene locus for *PD-L1* and *JAK2* expression is analyzed. (E) mRNA expression levels of PD-L1 are compared based on differing *JAK2* status. *H-JAK2*, high *JAK2* expression; *M-JAK2*, medium *JAK2* expression; *L-JAK2*, low*JAK2* expression. P<0.05 is significant.

Results

High JAK2 expression is associated with mRNA expression and gene amplification of PD-L1

To study the relationship between the mRNA expression of JAK2 and PD-L1 expression in thirty-one solid tumors, we investigated the TCGA databases, which include 9,315 tumor samples from thirty-one cancer types. The median log2-transformed mRNA expression values of JAK2 and PD-L1 were 8.2 and 4.8, respectively. The mRNA expression of JAK2and PD-L1 varied according to cancer type (P<0.0001; *Figure 1A,B*). Among the solid tumors, STAD and diffuse large B-cell lymphoma (DLBC) had the highest *JAK2* median values (9.3 and 9.2, respectively; *Figure 1A*), followed by kidney clear cell carcinoma (KIRC). In contrast, liver hepatocellular carcinoma and uveal melanoma (UVM) had the lowest *JAK2* median values (6.9 and 6.8, respectively; *Figure 1A*). As expected, the solid tumors with high *JAK2* expression, such as STAD and DLBC, showed high mRNA expression of PD-L1 (*Figure 1B*).

Because amplification of the gene locus for PD-L1 has been reported to serve as a good predictive biomarker of the response to anti-PD-1/PD-L1 therapy (19,20). The frequency of this amplification was analyzed in the various cancer types (*Figure 1C*). Ovarian serous cystadenocarcinoma (OV), head and neck squamous cell carcinoma (HNSC), sarcoma (SARC), and DLBC showed the highest proportion of amplification (*Figure 1C*). As in

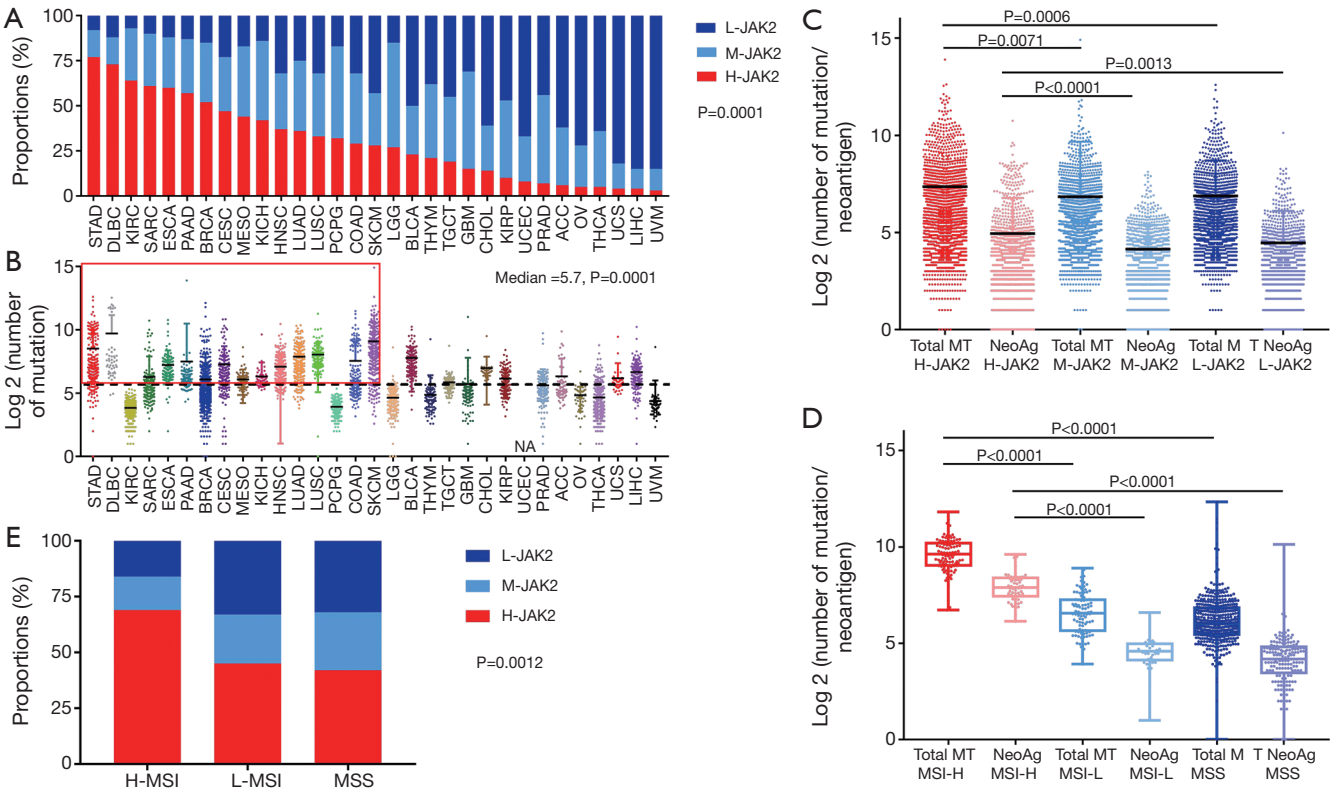

Figure 2 High Janus kinase 2 (*JAK2*) expression is associated with high mutational burdens, neoantigens, and MSI-H. (A,B) The distribution of different *JAK2* expression levels and mutation load in thirty-one cancer types is shown according to the analysis of The Cancer Genome Atlas (TCGA) database values. The values of mutation load are log2-transformed. (C) Total numbers of somatic mutations and neoantigens compared on the base of differing *JAK2* status in the cancer types. (D) Total numbers of somatic mutations and neoantigens compared based on differing microsatellite instability (MSI) status according to the cancer types from TCGA. (E) Fractions of different levels of *JAK2* expression compared according to differing MSI status. Total MT, total number of somatic mutations; NeoAg, number of neoantigens; MSI-H, microsatellite instability-high; MSI-L, microsatellite instability-low; MSS, microsatellite stability. P<0.05 is significant.

previous reports, the mRNA expression levels of JAK2 and PD-L1 were significantly correlated (r=0.5392, P<0.0001; *Figure 1D*). Both JAK2 and PD-L1 exhibited high mRNA expression levels under the status of gene locus amplification for PD-L1.

We also found that the mRNA expression of PD-L1 in the *H-JAK2* group was higher than that in *M-JAK2* or *L-JAK2* (P<0.0001; *Figure 1E*). The *H-JAK2* samples constituted a larger proportion in the group exhibiting gene locus amplification of PD-L1 than that in the non-amplified group (76.9% *vs.* 32.6%, P<0.0001; *Figure 1F*). The proportion of *M-JAK2* or *L-JAK2* was prominently lower in the amplification compared to the no-amplification (*Figure 1F*).

High JAK2 expression is associated with high mutational burden, neoantigen, and MSI-H

We sought to investigate the correlation between high *JAK2* expression and mutational burden. In every type of tumor, samples were divided according to their *JAK2* expression into three groups: high, medium, and low. The proportion of *H-JAK2* was quite high in STAD (76.6%) and DLBC (72.9%), but significantly low in liver hepatocellular carcinoma (LICH, 3.4%) and UVM (2.5%; *Figure 2A*). The proportion of *JAK2* expression differed in all tumors (P=0.0001; *Figure 2A*).

In addition, we compared the mutational burden of every tumor and discovered that tumors with *H-JAK2*, such as

STAD and DLBC, mostly bore high mutational burden (*Figure 2B*). UVM with *L-JAK2* showed low mutational burden. However, among *H-JAK2* tumors, kidney chromophobe (KICH) presented low mutation burden, whereas LICH, compared with other *L-JAK2* tumors such as OV and thyroid carcinoma (THCA), presented higher mutation burden (*Figure 2B*). We also discovered a clear association between mutational burden and the number of neoantigens (r=0.9386, P<0.0001; *Figure S1*). Further analysis linked *H-JAK2* to not only the highest mutational burden but also the highest emergence of neoantigens, compared with *M-JAK2* or *L-JAK2* in those tumors (*Figure 2C*).

MSI-H status indicates better response to immunotherapy, especially in COAD (21). We therefore examined the relationship between *JAK2* expression and MSI status. We found that COAD, ESCA, STAD, UCEC, and UCS showed changed MSI status. MSI-H tumors were loaded with the heaviest mutational burden (P<0.0001; *Figure 2D*), including STAD and COAD (P<0.0001; *Figure S2A,B*). Furthermore, the MSI-H group showed the highest proportion of *H-JAK2* compared to MSI-L and microsatellite stability (MSS) groups (P=0.0012; *Figure 2E*), especially in STAD and COAD (P<0.0001; *Figure S2C,D*).

High JAK2 expression is associated with tumor CYT activity and oncogenic viruses

To determine whether high *JAK2* expression is associated with CYT activity and oncogenic viruses, we sought to analyze the alterations of tumor CYT activity and oncogenic virus infection in *H-JAK2* patients. Using RNA-Seq data from thousands of TCGA solid tumor biopsies, we first found that *GZMA* and *PRF1* were tightly co-expressed in TCGA samples and exhibited a strong correlation across the TCGA database (r=0.8754, P<0.0001; *Figure 3A*). Patients with *H-JAK2* showed higher expression of *PRF1* and *GZMA* than those with *M-JAK2* and *L-JAK2* (P<0.0001; *Figure 3B,C*).

We next investigated the distribution of oncogenic virus infection including HPV, EBV, and HBV. Consistent with previous analysis of TCGA data, STAD exhibited the highest fraction of EBV infection (5.5%). HPV infection was most abundant in cervical cancer (55.6%), but also frequent in head and neck cancer (*Figure 3D*). HBV was primarily observed in liver samples (14.2%). Consistent with a previous report that demonstrated that oncogenic virus infection increased the CYT activity of a tumor (12), we found that the tumor CYT activity was notably associated with oncogenic viruses and that HPV or EBV positive samples demonstrated a high CYT expression (*Figure 3E*, *Figure S3*), whereas HBV positive samples showed a low CYT expression. Further investigation revealed that EBV positive samples featured the highest proportion of *H-JAK2*, whereas HBV positive samples had the lowest proportion of *H-JAK2* (P<0.001; *Figure 3F*).

High JAK2 expression is associated with tumor infiltrating CD8⁺CTLs

Central to the efficacy of immune checkpoint blockade is the requirement for immune cells to infiltrate into tumors (6). As tumor-infiltrating $CD8^+$CTLs mediate the antitumor response of immunotherapy, we aimed to discover the association between *H-JAK2* and *CD8A* expression. Notably, we found that there was a significant correlation between *CD8A* and interferon gamma (*IFNG*), and between *GZMB* and the mRNA expression of PD-1 (*Figure 4A,B,C*). Positive expression for each factor was defined as above-median expression. In addition, the *H-JAK2* group showed a large number of patients with tumor infiltrating $IFN\gamma^+CD8A^+$, $GZMB^+CD8A^+$, and $PD\text{-}1^+CD8A^+$ CTLs (*Figure 4A,B,C*). Patients with *M-JAK2* or *L-JAK2* showed lower mRNA expression of IFN-γ, GZMB, and PD-1 than *H-JAK2*, and patients with high *CD8A* expression encompassed a higher proportion of *H-JAK2* than those with medium or low *CD8A* expression (*Figure 4D*). The TCGA samples were divided equally into three groups according the RPKM values of *CD8A*. As expected, the samples with *H-CD8A* exhibited the highest proportion of *H-JAK2* (P<0.0001; *Figure 4E*).

Discussion

Based on TCGA dataset information, we classified thirty-one types of cancer into three groups according on their *JAK2* mRNA expression levels as assessed by RNA-Seq. The key finding of the current study consisted of the discovery that the mRNA expressions of PD-L1, mutational burdens, neoantigens, CYT activity, oncogenic viruses, and CD8+CTLs were significantly correlated with high *JAK2* expression. Thus, our results potentially indicate that *JAK2* might serve as a robust biomarker in Pan-Cancer; however, limited information was provided for guiding immunotherapy and biomarker strategies.

Although several studies have demonstrated that PD-L1 expression on the surface of tumor cells and immune cells

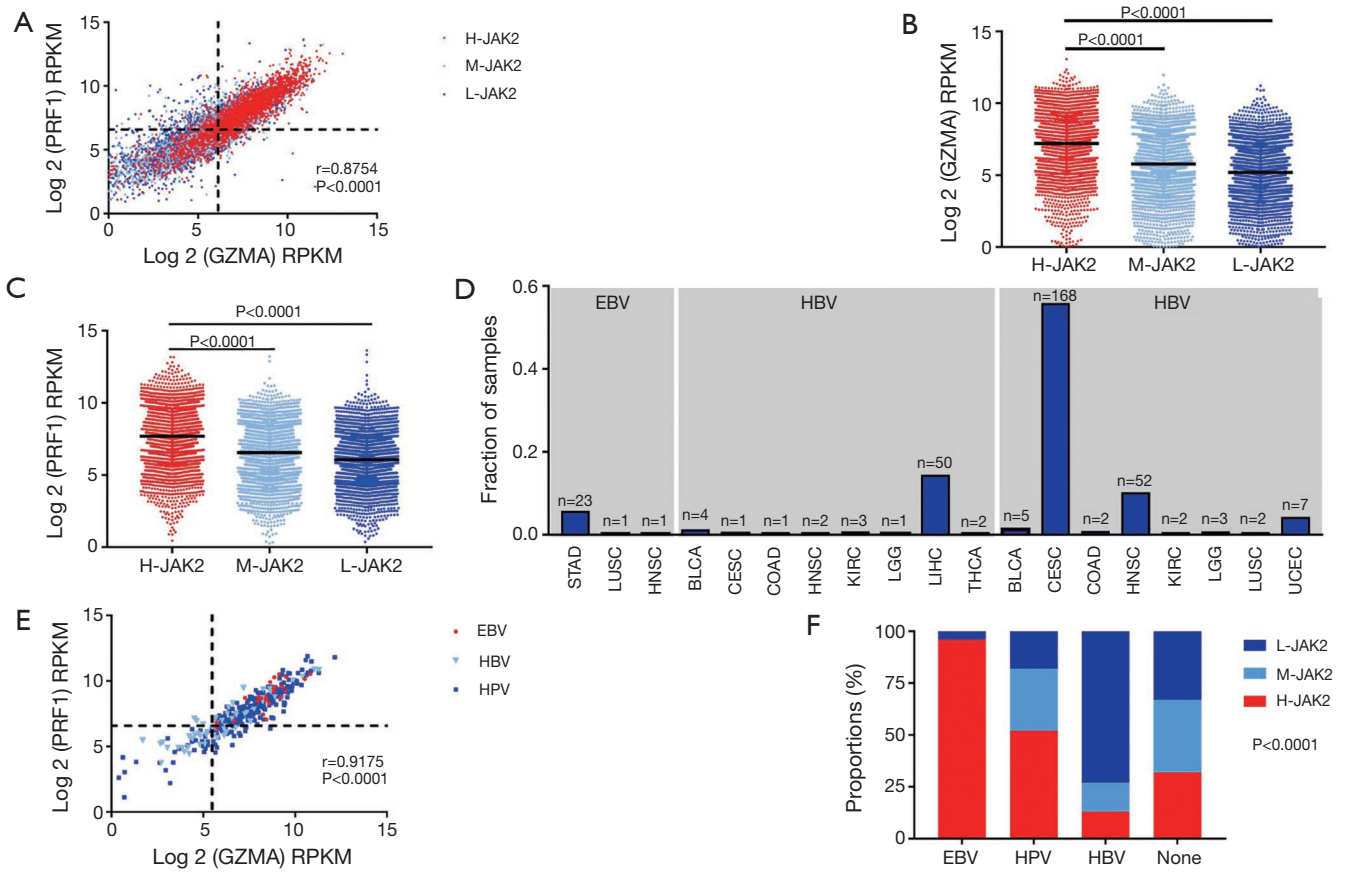

Figure 3 High Janus kinase 2 (*JAK2*) expression is associated with tumor cytolytic activity (CYT) activity and oncogenic viruses. (A) The association between differing *JAK2* status and CYT expression as measured by granzyme A (*GZMA*) and perforin 1 (*PRF1*) is shown. The values of *GZMA* and *PRF1* are log2-transformed. (B) The frequency of Epstein-Barr virus (EBV), hepatitis B virus (HBV), and human papilloma virus (HPV) infection across all cancer types is shown. (C) The association between CYT expression and different viral infections is plotted. (D,E) Expression levels of CYT are compared based on different *JAK2* levels. (F) The fractions of different *JAK2* expression levels are compared according to differing status of oncogenic viruses. CYT, cytolytic activity; HBV, hepatitis B virus; HPV, human papilloma virus; EBV, Epstein-Barr virus. P<0.05 is significant.

was a predictive biomarker of patient response to anti-PD-1/PD-L1 therapies in several cancer types (4,22), not all PD-L1-positive patients respond well to such treatments. In addition, the undefined optimal cutoff of PD-L1, such as 5% or 1%, and its diverse indication in different cancer types as well as adverse patient response to various anti-PD-1/PD-L1 drugs have limited the application of this immune therapy (23). Previous studies have clearly suggested that most human cancers, such as STAD, DLBC, ESCA, and lung adenocarcinoma (LUAD), present variable copy number gains of chromosome 9p24.1, a genomic region that includes the genes for PD-L1, PD-L2 (another ligand of PD-1), and JAK2, which activates the IFNγ/JAK/STAT pathway (15,24-26). The results of our study also confirmed that the mRNA expression levels of JAK2 and PD-L1 were prominently correlated. The amplification of the gene loci for JAK2 and PD-L1 was also highly consistent based on TCGA dataset information. In addition, recent studies have shown that the aberrant status of *JAK2* mutation led to a lack of PD-L1 expression upon IFNγ exposure mediated by an inability to signal through the IFNγ receptor pathway (17). Furthermore, *JAK2* loss-of-function alterations as noted in TCGA confer adverse outcomes in patients who showed a resistance to anti-PD-1/PD-L1 therapy (17,27).

The mutational burden varies among cancer types and is closely associated with the number of nonsynonymous mutations. Recent results have demonstrated that high

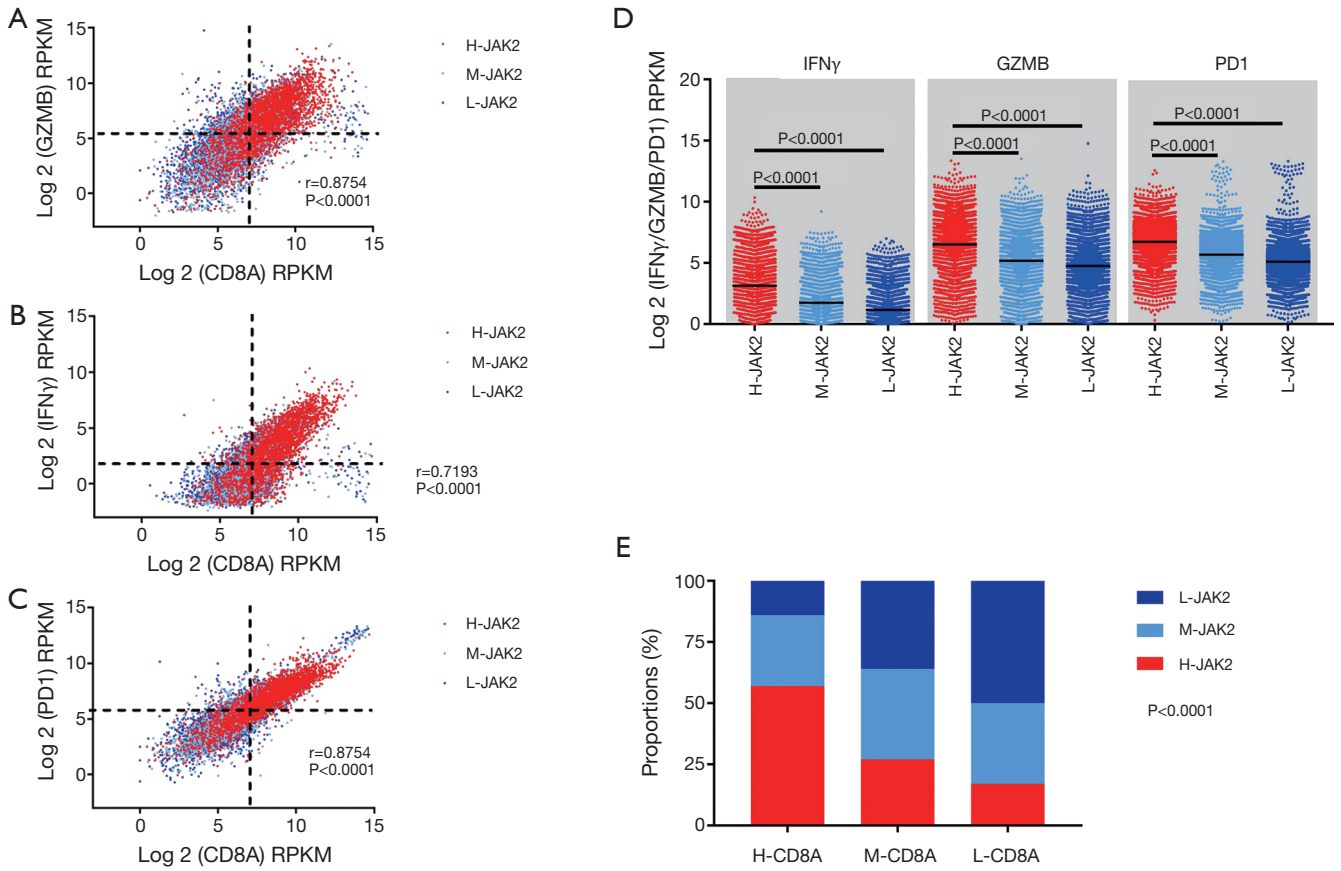

Figure 4 High Janus kinase 2 (*JAK2*) expression is associated with tumor infiltrating CD8$^+$ cytolytic T lymphocytes (CTLs). (A-C) The associations of CD8A expression with the mRNA expression of granzyme A (GZMA), IFN γ , and programmed cell death protein-1 (PD-1) are shown. The correlation between differing *JAK2* status and subtypes of CD8$^+$CTLs was analyzed. All mRNA expression values of GZMB, IFN γ , PD-1, and CD8A are log2-transformed. (D) The expression levels of GZMB, IFN γ , and PD-1 were compared based on differing *JAK2* status. (E) The frequency of differing *JAK2* status is compared within differing CD8A status. P<0.05 is significant.

mutational burden and nonsynonymous mutations improve the clinical outcome of anti-PD-1 antibody treatment (8,28,29). In our study, the cancer types with high proportion of *H-JAK2* such as STAD, DLBC, and ESCA were accompanied with high mutational burden. In contrast, a low proportion of *H-JAK2* generally occurred with low mutational burden in OV, THCA, and UVM. Notably, we found a prominent association between mutational burden and neoantigens. Theoretically, it would be feasible to calculate the interaction between a specific mutation and HLA genotype to predict the specific neoantigens (30). Furthermore, the number of neoantigens of the *H-JAK2* group was significantly higher than that of *M-JAK2* and *L-JAK2* over a total of thirty-one kinds of cancers. As shown by a recent study (28) and our report, MSI status was also correlated with mutational burden and neoantigens. In our results, the MSI-H samples displayed a higher proportion of *H-JAK2* than MSI-L and MSS. Previous findings discovered that patient MSI status was prominently associated with their response to immunotherapy (9,31,32). For example, patients with COAD and MSI-H benefit more from anti-PD-1/PD-L1 therapy, whereas the patients with MSI-L or MSS fail to respond (21).

In addition, another crucial issue related to treatment response is that some tumors are "inflamed" with effect or T cell infiltration whereas others are not. Growing evidence suggests that inflamed tumors respond more actively than non-inflamed tumors (33). As a key factor of inflammation, *JAK2* exhibited a significant association with tumor infiltrating CD8$^+$

CTLs as affirmed in this study. Furthermore, we found that not only PD-L1 but also immune molecules, such as IFNγ$^+$CD8A$^+$, GZMB$^+$CD8A$^+$, and PD-1$^+$CD8A$^+$, were prominently associated with high *JAK2* expression in the tumor microenvironment. In addition, viruses giving rise to a subset of inflamed malignancies are also known to activate high affinity antigen-specific CTLs against non-self-viral antigens (34-37). This phenomenon increases the immunogenicity of the tumor by activating the IFNγ pathway and leads to unregulated *JAK2* expression. Consistent with this phenomenon, tumors with oncogenic virus infection, such as HPV or EBV, showed high proportions of *H-JAK2*. Of note, samples with HBV infection exhibited a lower proportion of *H-JAK2* than uninfected samples. This indicated that the samples with HBV infection, especially those in hepatocellular carcinoma, were in an immunosuppressive state. Oncogenic virus infection increases the tumor CYT activity as measured by *GZMA* and *PRF1*, and was also found to be markedly correlated with *H-JAK2*.

Our study was limited considering the required clinical validation of *JAK2* cutoff values; however, the potential association between *H-JAK2* and several predicted biomarkers across most cancer types identified by using TCGA project database information should be highlighted. Our results were fundamentally consistent with previous findings, such as for STAD, DLBC, and LUAD, which showed relatively high proportion of *H-JAK2* and better response to anti-PD-1/PD-L1. These findings provide a reference for future preclinical and clinical studies regarding the application of *JAK2* expression toward the assessment of immuno-genomic features among cancer types.

In summary, analysis of TCGA samples has revealed that high *JAK2* expression was clearly associated with high mRNA expression and mutational burden of PD-L1, CD8$^+$CTLs, CYT expression, and oncogenic viral infection, which are likely good indicators for the response to anti-PD-1/PD-L1 therapy. Our data thus support the combination of *H-JAK2* and multiple biomarker assays, and may facilitate the discovery of new anti-PD-1/PD-L1 therapeutic strategies that could screen a cohort of patients who may acquire greater benefit from immunotherapy.

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Footnote

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at http://dx.doi.org/10.21037/tcr.2018.04.09). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). Institutional ethical approval and informed consent were waived.

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