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The Role of *EPHA3* Mutation in the Prognosis of Non-Small Cell Lung Cancer Patients Receiving Immunotherapy

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Abstract

Immune Checkpoint Inhibitors (ICIs) have changed the treatment mode of Non-Small Cell Lung Cancer (NSCLC) patients, but precise biomarkers are still needed to screen out those could benefit from ICIs. *EPHA3* is the gene that codes for the *Eph receptor A3* and has been found to be associated with lung cancer, but the relationship between *EPHA3* and ICIs still need to be explored. In our study, data of 344 NSCLC patients receiving ICIs and 954 NSCLC patients treated without ICIs were downloaded from the Memorial Sloan Kettering Cancer Center (MSKCC) database and The Cancer Genome Atlas (TCGA) database respectively. Patients were divided into *EPHA3*-mutant type (*EPHA3*-Mut) group and *EPHA3*-wild type (*EPHA3*-Wt) group by *EPHA3* mutation status. Kaplan-Meier survival analysis found that the *EPHA3*-Mut group (n=36) have got higher Overall Survival (OS) rates than the *EPHA3*-Wt group (n=308) (median OS: 3 years (95% Confidence Interval (CI)=1 to not reached) *vs.* 0.917 years (95% CI=0.75 to 1.17, p=0.025) in the MSKCC cohort, while differences of OS (p=0.083) in the TCGA cohort have not been observed. Besides, *EPHA3* mutation was related to higher Tumor Mutation Burden (TMB) (p<0.0001), elevated Neoantigen Load (NAL) (p<0.0001) and greater mutation rate in the DNA Damage Response (DDR) pathways. *EPHA3*-Mut group showed higher $CD8^+$ T cells (p<0.05) an Natural Killers (NK) cells (p<0.01) infiltration. Gene Set Enrichment Analysis (GSEA) showed that several immune response-related pathways were up-regulated in the *EPHA3*-Mut group. According to the study, *EPHA3* mutation may be related with the effectiveness of ICIs in NSCLC patients.

Keywords: Immunotherapy; Immuno-checkpoint inhibitors; Non-small cell lung cancer; *EPHA3*; Immune

Introduction

ICIs have altered the treatment mode of NSCLC, some patients with NSCLC achieved long-term survival through immunotherapy [1]. However, there are still majority of patients who cannot significantly benefit from immunotherapy [2]. Therefore, we urgently need some biomarkers to screen out patients who could benefit from immunotherapy. Up to now, there are some potential biomarkers such as PD-L1 expression and Tumor Mutation Burden (TMB) already been found out to predict the curative effect of ICIs [3]. However, in some studies, these potential biomarkers cannot accurately predict the curative effect of immunotherapy, for example, some scholars found that NSCLC patients with a PD-L1 expression level of 5% or more did not reach longer Progression-Free Survival (PFS) after treated with Nivolumab [4]. Thus, more precise biomarkers of ICIs remain to be explored. Receptor Tyrosine Kinases (RTKs) is the largest class of enzyme linked receptor proteins, many types of RTKs play a critical part in the development and growth of tumors and are related with curative effect of ICIs. For instance: Epidermal Growth Factor (EGF) Receptor (EGFR) pathway activation could reduce the PD-L1 expression in NSCLC patients and is correlated with immunosuppression, Fibroblast Growth Factor (FGF) Receptor 4 (FGFR4) was one of the main targets for down-regulation of PD-L1 *in vitro*, while FGFR2 promotes PD-L1 expression in colorectal cancer through JAK/STAT3 signaling pathway, interruption of gastrin at the Cholecystokinin (CCK) receptor may alter tumor immune cells, which may could affects the efficacy of immunotherapy [5-8]. Eph receptor family is the largest known family of RTKs, according to their extracellular domains, Eph receptors are divided into two subgroups, Eph receptor A (EphA) and Eph receptor B (EphB), EphA is consisted of 9 members, while EphB is consisted of 5 members [9]. *EPHA3* is the gene that codes for the *Eph receptor A3*, it has been found to be associated with the development of lung adenocarcinoma and thus, we speculated whether *EPHA3* has effect on the curative effect of ICIs.

Therefore, we conducted a comprehensive analysis through an immunotherapy cohort (MSKCC cohort), and TCGA cohort to determine whether *EPHA3* is associated with the curative effect of ICIs on NSCLC patients [10]. The result showed that *EPHA3* mutation could improve NSCLC patients' OS treated with ICIs. Besides, *EPHA3* mutation is connected with greater immune cell soakage in the Tumor Immune Microenvironment (TIME), higher tumor antigenicity, more gene mutations in the DNA Damage Response (DDR) pathways and more activation of immune-related pathways in NSCLC patients. These results imply that *EPHA3* mutation may act as predictive biomarker of immunotherapy efficacy in patients with NSCLC.

Materials and Methods

Clinical cohorts and genome characteristics

To find out the relationship between *EPHA3* mutation and ICIs, we downloaded previously published immunotherapy cohort

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from cBioPortal, as discovery cohort (n=344, *EPHA3*-Mut group *vs.* *EPHA3*-Wt group=36:308), all patients in the cohort were NSCLC patients recruited from MSKCC who were treated with PD-1 or PD-L1 inhibitors [10]. We also downloaded somatic mutation of NSCLC patients who had not treated with ICIs form TCGA database from the Genomic Data Commons (GDC) portal, after excluding the samples with incomplete information, there are 954 (*EPHA3*-Mut *vs.* *EPHA3*-Wt =113:841) samples. Targeted Next-Generation Sequencing (NGS) was used for the analysis of the somatic mutation data from the MSKCC cohort. Maftools package in the R was used to visualize the top 20 most commonly mutated genes and clinical features. Besides, the co-mutation and mutually exclusive mutation of top 25 most commonly mutated genes in MSKCC cohort was explored through Maftools package.

Survival analysis

MSKCC cohort and TCGA cohort are divided to *EPHA3*-Mut group and *EPHA3*-Wt group according to *EPHA3* mutation status. Kaplan-Meier survival curves analyses were used to compare OS between *EPHA3*-Mut group and *EPHA3*-Wt group in both two cohorts. Besides, we performed univariate and multivariate cox regression analysis to explore the relationship between patients' OS and some frequently mutated genes, including *EPHA3*, as well as clinical features of NSCLC patients in the MSKCC cohort. In this analysis, TMB was divided into two groups according to dividing line of 10 Mut/Mb [11,12].

Analysis of tumor antigenicity

To investigate whether *EPHA3* mutation has an effect on tumor antigenicity, we explored the level of TMB, Neoantigen Load (NAL) as well as mutations in DDR pathways in both *EPHA3*-Mut group and *EPHA3*-Wt group. The number of non-synonymous somatic mutations is divided by 38 Mb to obtain the TMB value. NAL data and mutation data in DDR pathways were obtained from researches of Thorsson et al., and Knijnenburg et al., respectively [13,14].

Analysis of immune cell soakage was performed

To explore the connection of *EPHA3* mutation and immune soakage, we used the data of 22 kinds of immune cells' signature matrix obtained from CIBERSORTx, to explore the feature of immune cell soakage in the TIME of patients in the TCGA cohort, we then compared the immune cell soakage status of *EPHA3*-Mut group and *EPHA3*-Wt group [15]. Online analytical tool CIBERSORTx was applied to conduct the analysis.

Gene set enrichment analysis

To further study the biological pathways related to *EPHA3*, we downloaded hallmark gene sets and ontology gene sets from Molecular Signatures Database (MSigDB) of the Broad Institute [16]. Then, clusterProfiler package of R was used for the GSEA. Pathways with $p<0.05$ were considered significantly different.

Statistical analysis

Fisher's exact test was used to analyse co-mutation and mutually exclusive mutation of top 25 most commonly mutated genes in MSKCC cohort. Kaplan-Meier method with the log-rank test were applied to graph survival curves. Univariate and multivariate cox regression analysis were conducted to determine the potential of *EPHA3* mutation, other common mutated gene mutations and clinical features to predict the efficacy of immunotherapy in the MSKCC cohort. Mann-Whitney U test was used to compare the differences of TMB and NAL between *EPHA3*-Mut group and *EPHA3*-Wt group. Gene mutations of DDR pathways in *EPHA3*-Mut group and *EPHA3*-Wt group in TCGA cohort were compared using Chi-square test. All statistical tests were two-sided.

Results

Clinical and genetic characteristics of patients

Our discovery cohort is consisted of 344 NSCLC patients receiving ICIs, 119 (35.5%) of them were over 71 years old, 178 (51.7%) of them were men, 166 (48.3%) of them were women, 268 (77.9%) were lung adenocarcinoma, 44 (12.8%) were lung squamous cell carcinoma, other 32(9.3%) of them were other kind of NSCLC. The median TMB was 7.76 mutations/Mb (range, 0-96.5 mutations/Mb). The waterfall plot was used to demonstrate these genes whose mutation rate was in the top 20, as well as clinical features of the NSCLC patients in MSKCC cohort, as shown in the plot, *EPHA3* was the seventh most frequently mutated gene. Besides, Gene interaction analysis of MSKCC cohort shows that *EPHA3* tended to co-mutate with *KRAS*, *ZFHX3*, *PTPRT* and *RBM10* ($p<0.05$) (Figures 1a and 1b).

The relationship of OS and *EPHA3* mutation in NSCLC patients receiving ICIs

To analyse the contribution of *EPHA3* to prognosis of NSCLC patients treated with ICIs, we conducted survival analyses for MSKCC and TCGA cohorts. As showed in the Kaplan-Meier analysis, in the MSKCC cohort, *EPHA3*-Mut group had observably longer OS ($p=0.025$). The median OS was 3 years (95% CI=1 to not reached) in *EPHA3*-Mut group *vs.* 0.917 years (95% CI=0.75-1.17) in *EPHA3*-Wt group. Compared with MSKCC cohort, there is no significant differences of OS ($p=0.083$) between *EPHA3*-Mut group and *EPHA3*-Wt group in the TCGA cohort. The median OS was 3.98 years (95% CI=3.3 to not reached) in *EPHA3*-Mut group *vs.* 3.53 years (95% CI=3.03-4.5) in the group of *EPHA3*-Wt group. Univariate cox regression analysis showed that TMB (HR=0.714, 95% CI=0.530-0.961, $p=0.026$) and *EPHA3* (HR=0.57, 95% CI=0.342-0.949, $p=0.031$) is associated with longer OS. While multivariate cox regression analysis showed that the effect of *EPHA3* on OS did not reach statistical significance (HR=0.635, 95% CI=0.372-1.086, $p=0.097$) (Figures 2a-2d).

EPHA3 mutation is relative to the enhancement of tumor immunogenicity and gene mutations in DDR pathways

Tumor immunogenicity is related to the efficacy of immunotherapy. To some degree, TMB and NAL could reflect the immunogenicity of tumor and are related to the curative effect of ICIs [17,18]. To find out the relationship between *EPHA3* mutation and tumor immunogenicity, we investigated the differences of TMB and NAL between *EPHA3*-Mut group and *EPHA3*-Wt group. In both MSKCC cohort ($p<0.001$) and TCGA cohort ($p<0.0001$), TMB of *EPHA3*-Mut group was higher than *EPHA3*-Wt group. We also observed that *EPHA3*-Mut group shows significantly higher NAL compared to *EPHA3*-Wt group in TCGA cohort ($p<0.0001$).

Some reports showed that some mutations in DDR pathways could represent genomic instability and may be related to better outcomes of immunotherapy [19]. We compared mutations in 9 DDR pathways between *EPHA3*-Mut and *EPHA3*-Wt group in TCGA cohort. As expected, in *EPHA3*-Mut group, the mutation rates were higher in 7 DDR pathways, including Base Excision Repair (BER),

Homologous Recombination (HR), Nucleotide Excision Repair (NER), Nonhomologous End-Joining (NHEJ), Mismatch Repair (MMR), Translesion DNA Synthesis (TLS) and Fanconi Anemia (FA) (Figures 3a-3d).

These results indicated that *EPHA3* mutation is correlated with the enhancement of tumor immunogenicity and more gene mutations in DDR pathways, which suggested a better prognosis of NSCLC patients receiving ICIs.

EPHA3 mutation is correlated with immune soakage

The immune status of the TIME also has effects on the efficacy of immunotherapy [20]. To investigate how the *EPHA3* mutation affects TIME, we used the immune cell signature matrix to study the differences of soakage of immune cells between *EPHA3*-Mut group and *EPHA3*-Wt group in TCGA cohort. As expected, in *EPHA3*-Mut group, CD8+ T cells ($p<0.05$) as well as activated NK cells ($p<0.01$) were found obviously increased (Figure 4).

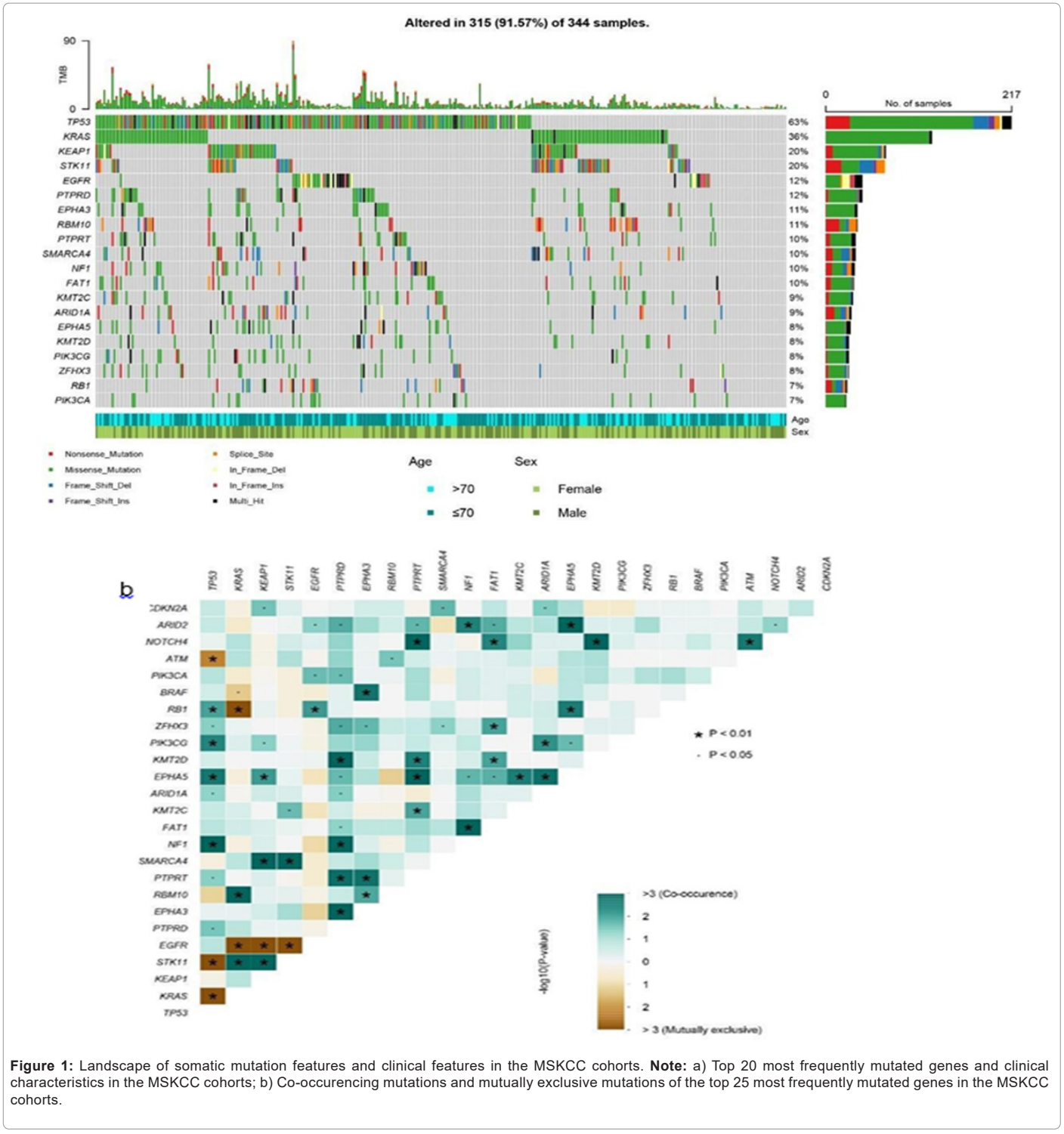

Figure 1: Landscape of somatic mutation features and clinical features in the MSKCC cohorts. **Note:** a) Top 20 most frequently mutated genes and clinical characteristics in the MSKCC cohorts; b) Co-occurencing mutations and mutually exclusive mutations of the top 25 most frequently mutated genes in the MSKCC cohorts.

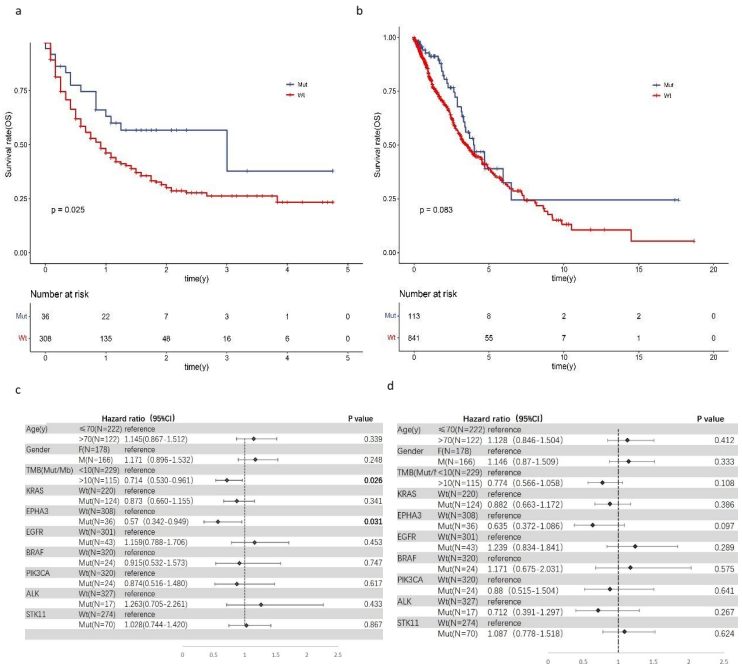

Figure 2: Association between *EPHA3* mutation and prognosis of NSCLC patients. **Note:** a) Kaplan-Meier curves comparing OS of patients with or without *EPHA3* mutation in the MSKCC cohort; b) Kaplan-Meier curves comparing OS of patients with or without *EPHA3* mutation in the TCGA cohort; c) Forest plot displaying the results of univariate cox proportional-hazard regression analysis of *EPHA3* mutation and other common TKI-sensitive gene mutations in the MSKCC cohort; (d) Forest plot displaying the results of multivariate cox proportional-hazard regression analysis of *EPHA3* mutation and other common TKI-sensitive gene mutations in the MSKCC cohort.

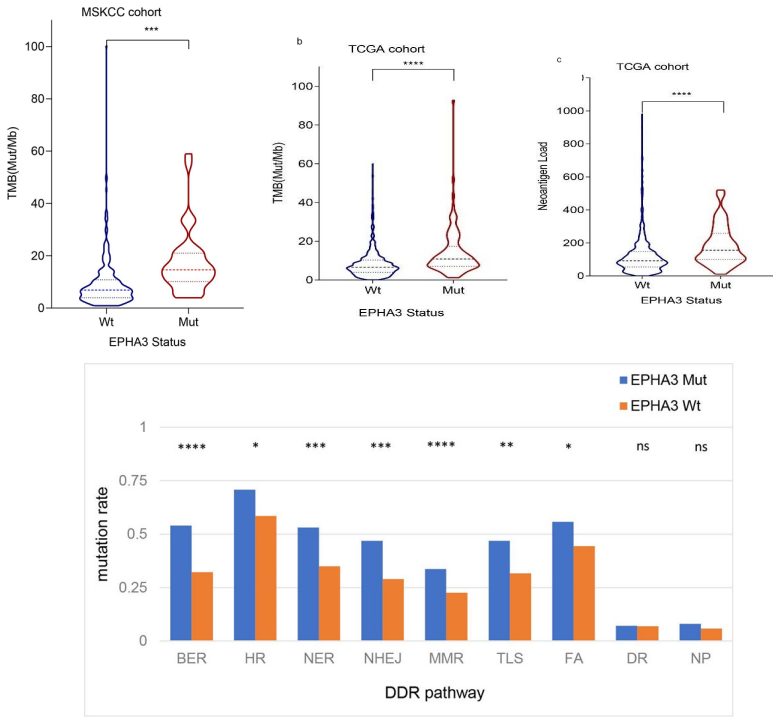

Figure 3: Association of *EPHA3* mutation with tumor mutation burden, neoantigen load and mutations in DNA Damage Repair (DDR) pathways in NSCLC patients. **Note:** (a,b) *EPHA3* mutated patients had a markedly higher TMB (number of mutations per Mb) in both the MSKCC-IO cohort and the TCGA cohort; (c) Comparison of NAL (number of neoantigen per Mb) between the *EPHA3*-Mut and *EPHA3*-Wt group tumors in the TCGA cohorts (Mann-Whitney U test); (d) Comparison of mutation rate in the DDR pathways between the *EPHA3*-Mut group and *EPHA3*-Wt group in the TCGA cohort (Chi-square test); *p<0.05; **p<0.01; ***p<0.001;****p<0.0001; ns: No significance.

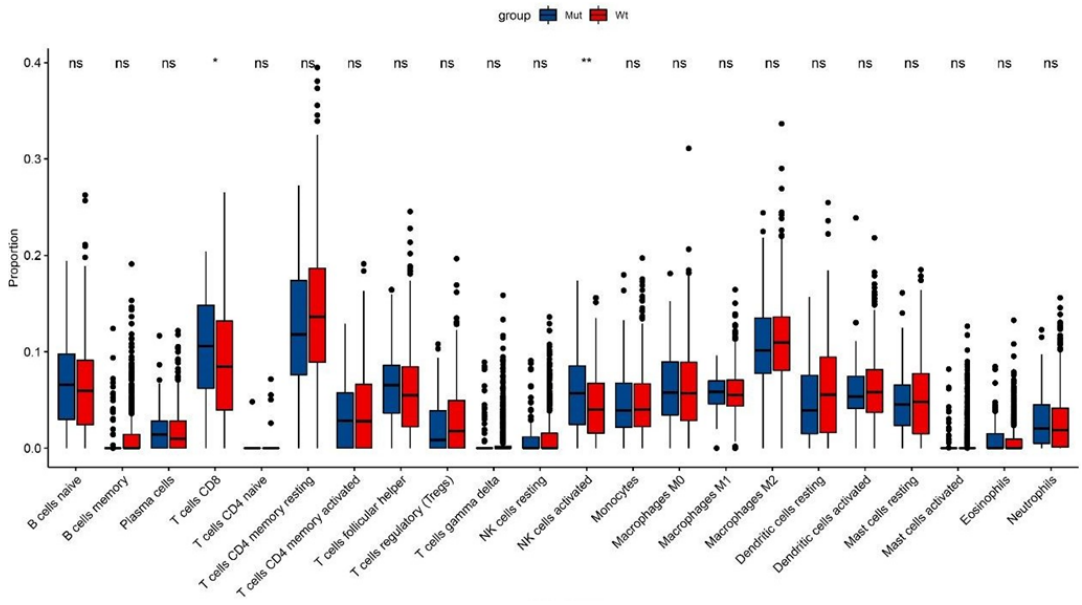

Figure 4: Association of *EPHA3* mutation with immune infiltration. Infiltration frequencies of 22 types of immune cells in the *EPHA3*-Mut group and *EPHA3*-Wt group in the TCGA cohort. **Note:** *p<0.05; **p<0.01; ns: No significance.

EPHA3 mutation is correlated with changes in some tumor-related biological pathways

In order to identify whether *EPHA3* mutation acts on tumor-related biological pathways, we performed GSEA on TCGA cohort and compared the results of *EPHA3*-Mut group and *EPHA3*-Wt group. As shown in Supplementary Figure 1, a number of immune-related pathways were markedly up-regulated in the *EPHA3*-Mut group, such as activation of immune response pathway, B cell activation pathway, INF-γ response. In contrast, some pathways such as mTORC1 signaling pathway and MYC targets V1 pathway were down-regulated in the *EPHA3*-Mut group. These results showed that *EPHA3* mutation has positive effect on immune soakage and immune response.

Discussion

Through univariate cox regression analysis and Kaplan-Meier survival analysis, we observed that *EPHA3* mutation has a favorable effect on OS of NSCLC patients receiving ICIs, while no effect on patients who treated without ICIs. Although the multivariate cox regression analysis of the effect of *EPHA3* on OS did not reach statistical significance, there is still an obvious tendency of *EPHA3* to prolong OS of NSCLC patients treated with ICIs (HR=0.635), the reason for not reaching statistical significance (p=0.097) may be that the sample size was insufficient to detect the significance of the difference. Through gene interaction analysis, we found that *EPHA3* co-mutated with several genes that may have potential effect for immunotherapy. Then, our investigation on tumor immunogenicity discovered that there are higher TMB, NAL, as well as more mutations in DDR pathways in *EPHA3*-Mut group. In addition, increased soakage of immune cells in Tumor Microenvironment (TME) were found in EPHA-Mut group. GSEA analysis showed that some tumor-related biological pathways, including immune response-related pathways were up-regulated in *EPHA3*-Mut group, while some pathways were down-regulated. These findings proved that *EPHA3* may act as a potential prognostic biomarker for immunotherapy. Eph receptors are closely related to cellular repulsion, adhesion and other activities, *EPH* gene mutations are related to tumorigenesis, tumor immunity and tumor angiogenesis [21,22]. Recently, some studies indicated that Eph receptors could suppress the immune response by modulate innate and adaptive immunity in the TME [23]. Thus, researchers hypothesized that we may inhibit the function of these receptors to raise immune response and enhance the efficacy of ICIs. In recent studies, *EPHA5* and *EPHA7* have been found to be related to immunotherapy efficacy in patients with lung cancer [24,25]. Besides, researchers have found that *EPHA3* was one of the most commonly mutated genes in lung cancer and may inhibit the formation of lung adenocarcinoma [26]. However, the relationship between *EPHA3* mutation and ICIs has not been found out yet. Our study is the first to represent the association between the efficacy of ICIs and *EPHA3* mutation in NSCLC patients, the result showed that among NSCLC patients receiving ICIs, those with *EPHA3* mutation tend to get longer OS. Our study enriched the group of ICIs' prognostic biomarkers, strengthened the connection between Eph family and immunotherapy. In our study, *EPHA3* tended to co-mutate with *KRAS*, *ZFHX3*, *PTPRT* and RBM10. *ZFHX3* mutation and *PTPRT* mutation have been found as a protective biomarker for immunotherapy on NSCLC patients [27,28]. *RBM10* deficiency was found been associated with higher TMB and PD-L1 expression in NSCLC patients, that means *RBM10* may affect the prognosis of patients using ICIs. Several reports also showed that tumors with *KRAS* mutated showed higher TMB and patients with *KRAS* mutated tended to benefit more from PD-1 inhibitors [29-31]. These findings support our hypothesis that *EPHA3* mutation may has a positive impact on the efficacy of immunotherapy in NSCLC patients. ICIs kill tumor cells based on their immunogenicity, which is primarily determined by tumor antigenicity and antigen presentation efficiency [32]. Antigens enable the immune system to distinguish body's own tissues and cancer cells. Neo-antigens are derived from about 10% of the non-synonymous somatic mutations and play an important role in antitumor response, for they are main targets of T-cell-mediated antitumor immunity [33,34]. In this study, *EPHA3*-Mut group showed higher TMB and NAL level, indicating that *EPHA3* mutation may be associated with better prognosis of NSCLC patients receiving immunotherapy. Some studies reported that patients

with mutations in DDR pathways tend to have higher TMB and NAL level, and might also result in high Microsatellite Instability (MSI-H), which means a higher immunogenicity of tumor [35]. It is also reported that mutations in DDR pathway in tumors directly affect the soakage of immune cells at the TIME, thus affecting the efficacy of ICIs [36-38]. Researchers found that gene mutations in DDR pathways may serve as a reliable biomarker to evaluate the efficacy of ICIs in clinical application [39]. In this study, we found that *EPHA3*-Mut group showed more mutations in DDR pathway, which means better prognosis in ICIs. The analysis of immune cell soakage in the TIME of NSCLC patients in the MSKCC cohort showed that $CD8^+$ T cells and activated NK cells were significantly increased in the *EPHA3*-Mut group. On the one hand, tumor microenvironment can induce the expression of PD-1 in $CD8^+$T cells as well as NK cells, which can bind to PD-L1 on the surface of tumor cells, thus assisting tumor cells in immune escape, that makes $CD8^+$ T cells and NK cells become the main target of PD-1 inhibitors [40,41]. On the other hand, activated $CD8^+$ T lymphocytes cells are the primary effector cells of antitumor immunity in the body, NK cells also take a significant part in tumor clearance, deficit of NK cells leads to an increased risk of cancer development, thus, the degree of $CD8^+$ T cells and NK cells' soakage in the TIME has a significant impact on tumor immunotherapy [42,43]. In our study, GSEA analysis showed that some immune-related pathways were upregulated, while E2F targets, MYC targets V1 and mTORC1 signaling pathways are downregulated in *EPHA3*-Mut group. E2F is an important transcription factor involved in carcinogenesis [44]. There are researches reported that there is a higher expression of E2F in NSCLC tumors, besides, overexpression of E2F1 and E2F2 was observably related to poor prognosis of patients [45]. MYC is a transcription factor with a wide range of roles, which can regulate a variety of cell activities including cell differentiation and proliferation through a variety of pathways. Scholars found that overexpression of MYC obviously reduced the populations of $CD3^+$, CD8+ T and disabled T cell soakage in the TIME in Triple-negative breast cancer [46]. Mammalian Target of Rapamycin (mTOR) is an important regulator of cell growth and proliferation as well as a central regulator of immune response, mainly acting through two multi-protein complexes, mTORC1 and mTORC2 [47,48]. It has been reported that mTOR plays an important part in the modulation of immune responses, it could regulate a variety of functions of professional antigen-presenting cells and also play an important role in regulatory T cells and effector T cells [49]. Some scholars identified that the dysfunction of immune cells is related to down regulation of proliferative signals (MYC targets, E2F targets, mTORC1 signaling) [50]. These findings indicated that *EPHA3* mutation has an effect on immune function and may further affect the efficacy of ICIs. The present study had several shortcomings. Firstly, this is a retrospective study, it may induce bias to this study. Then, the sample size of MSKCC cohort is limited, more cases and experiments are needed to confirm the results. Thirdly, due to the lack of relevant gene mutation information in clinical patients, the findings of this study lack clinical validation. Finally, due to the lack of detailed gene expression data in the MSKCC cohort, we were unable to verify the relationship of *EPHA3* mutation and immune cell soakage of tumor immune microenvironment and the gene mutation rate in the DDR pathways in the MSKCC cohort.

Conclusion

As shown in this study, *EPHA3* mutation may be a potential prognosis biomarker of NSCLC patients receiving ICIs. *EPHA3* mutation had been proved to have relationship with longer OS, more abundant immune cell soakage and stronger tumor antigenicity in patients treated with ICIs. However, it is necessary to deepen the research on the molecular mechanism of how *EPHA3* mutation works on immunotherapy and further preclinical and prospective clinical studies are needed to assess the clinical potential of *EPHA3* mutation as a biomarker to predict the prognosis of NSCLC patients those who receiving ICIs.

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Availability of Data and Material

The datasets used and analyzed during the current study are available from the Memorial Sloan Kettering Cancer Center database and The Cancer Genome Atlas database.

Author Agreements

All authors have seen and approved the final version of the manuscript being submitted. We warrant that the article is the authors' original work, hasn't received prior publication and isn't under consideration for publication elsewhere.

Ethical Approval

This study has been approved by the Ethics Committee of the Second Affiliated Hospital of Harbin Medical University, including ethical approval, statement of informed consent and exemption of informed consent. (Ethical review approval number: YJSKY2022-526).

Publication Consent

In this study, no information was available to identify the subjects and informed consent could not be obtained.

Declaration of Interest Statement

We declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Author Contributions

Qin Qin: Sorted out the research ideas, responsible for statistical drawing, and the main writer of the paper. Di Wang: Provide important guidance in solving difficult or complex problems in an article. Meng Ma and Jing Ai: Responsible for data collection and correction of grammar. Lili Deng: The main designer of the study and also responsible for the final review of the paper. All authors read and approved the final manuscript.

References

1. Gubin MM, Zhang X, Schuster H, Caron E, Ward JP, et al. (2014) Checkpoint blockade cancer immunotherapy targets tumour-specific mutant antigens. Nature 515:577-581.
2. Carbognin L, Pilotto S, Milella M, Vaccaro V, Brunelli M, et al.

(2015) Differential activity of nivolumab, pembrolizumab and mpdl3280a according to the tumor expression of Programmed Death-Ligand-1 (PD-L1): Sensitivity analysis of trials in melanoma, lung and genitourinary cancers. PLoS One 10:e0130142.

3. Keenan TE, Burke KP, van Allen EM (2019) Genomic correlates of response to immune checkpoint blockade. Nat Med 25:389-402.

4. Carbone DP, Reck M, Paz-Ares L, Creelan B, Horn L, et al. (2017) First-line nivolumab in stage IV or recurrent non-small-cell lung cancer. N Engl J Med 376:2415-2426.

5. Akbay EA, Koyama S, Carretero J, Altabef A, Tchaicha JH, et al. (2013) Activation of the PD-1 pathway contributes to immune escape in EGFR-driven lung tumors. Cancer Discov 3:1355-1363.

6. Yi C, Chen L, Lin Z, Liu L, Shao W, et al. (2021) Lenvatinib targets FGF receptor 4 to enhance antitumor immune response of anti-programmed cell death-1 in HCC. Hepatology 74:2544-2560.

7. Li P, Huang T, Zou Q, Liu D, Wang Y, et al. (2019) FGFR2 promotes expression of PD-L1 in colorectal cancer via the JAK/STAT3 signalling pathway. J Immunol 202:3065-3075.

8. Osborne N, Sundseth R, Burks J, Cao H, Liu X, et al. (2019) Gastrin vaccine improves response to immune checkpoint antibody in murine pancreatic cancer by altering the tumor microenvironment. Cancer Immunol Immunother 68:1635-1648.

9. Gale NW, Holland SJ, Valenzuela DM, Flenniken A, Pan L, et al. (1996) Eph receptors and ligands comprise two major specificity subclasses and are reciprocally compartmentalized during embryogenesis. Neuron 17:9-19.

10. Samstein RM, Lee CH, Shoushtari AN, Hellmann MD, Shen R, et al. (2019) Tumor mutational load predicts survival after immunotherapy across multiple cancer types. Nat Genet 51:202-206.

11. Karamitopoulou E, Andreou A, Wenning AS, Gloor B, Perren A, et al. (2022) High Tumor Mutational Burden (TMB) identifies a microsatellite stable pancreatic cancer subset with prolonged survival and strong anti-tumor immunity. Eur J Cancer 169:64-73.

12. Ready N, Hellmann MD, Awad MM, Otterson GA, Gutierrez M, et al. (2019) First-line nivolumab plus ipilimumab in advancednon-small-cell lung cancer (CheckMate 568): Outcomes by programmed death ligand 1 and tumor mutational burden as biomarkers. J Clin Oncol 37:992-1000.

13. Thorsson V, Gibbs DL, Brown SD, Wolf D, Bortone DS, et al. (2018) The immune landscape of cancer. Immunity 48:812-830.

14. Knijnenburg TA, Wang L, Zimmermann MT, Chambwe N, Gao GF, et al. (2018) Genomic and molecular landscape of DNA damage repair deficiency across the cancer genome atlas. Cell Rep 23:239-254.

15. Newman AM, Liu CL, Green MR, Gentles AJ, Feng W, et al. (2015) Robust enumeration of cell subsets from tissue expression profiles. Nat Methods 12:453-457.

16. Subramanian A, Tamayo P, Mootha VK, Mukherjee S, Ebert BL, et al. (2005) Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. Proc Natl Acad Sci USA 102:15545-15550.

17. Chan TA, Yarchoan M, Jaffee E, Swanton C, Quezada SA, et al. (2019) Development of tumor mutation burden as an immunotherapy biomarker: Utility for the oncology clinic. Ann Oncol 30:44-56.

18. Matsushita H, Sato Y, Karasaki T, Nakagawa T, Kume H, et al. (2016) Neoantigen load, antigen presentation machinery and immune signatures determine prognosis in clear cell renal cell carcinoma. Cancer Immunol Res 4:463-471.

19. Jiang M, Jia K, Wang L, Li W, Chen B, et al. (2021) Alterations of DNA damage response pathway: Biomarker and therapeutic strategy for cancer immunotherapy. Acta Pharm Sin B 11:2983-2994.

20. Zhao Y, Cao Y, Chen Y, Wu L, Hang H, et al. (2021) B2M gene expression shapes the immune landscape of lung adenocarcinoma and determines the response to immunotherapy. Immunology 164:507-523.

21. Brantley-Sieders D, Schmidt S, Parker M, Chen J (2004) Eph receptor tyrosine kinases in tumor and tumor microenvironment. Curr Pharm Des 10:3431-3442.

22. Pasquale EB (2010) Eph receptors and ephrins in cancer: Bidirectional signalling and beyond. Nat Rev Cancer 10:165-180.

23. Janes PW, vail ME, Ernst M, Scott AM (2021) Eph receptors in the immunosuppressive tumor microenvironment. Cancer Res 81:801-805.

24. Huang W, Lin A, Luo P, Liu Y, Xu W, et al. (2021) *EPHA5* mutation predicts the durable clinical benefit of immune checkpoint inhibitors in patients with lung adenocarcinoma. Cancer Gene Ther 28:864-874.

25. Zhang Z, Wu HX, Lin WH, Wang ZX, Yang LP, et al. (2021) *EPHA7* mutation as a predictive biomarker for immune checkpoint inhibitors in multiple cancers. BMC Med 19:26.

26. London M, Gallo E (2020) Critical role of *EphA3* in cancer and current state of *EphA3* drug therapeutics. Mol Biol Rep 47:5523-5533.

27. Zhang J, Zhou N, Lin A, Luo P, Chen X, et al. (2021) *ZFHX3* mutation as a protective biomarker for immune checkpoint blockade in non-small cell lung cancer. Cancer Immunol Immunother 70:137-151.

28. Wang X, Wu B, Yan Z, Wang G, Chen S, et al. (2021) Association of PTPRD/*PTPRT* mutation with better clinical outcomes in NSCLC patients treated with immune checkpoint blockades. Front Oncol 11:650122.

29. Liu B, Wang Y, Wang H, Li Z, Yang L, et al. (2021) *RBM10* deficiency is associated with increased immune activity in lung adenocarcinoma. Front Oncol 11:677826.

30. Dong ZY, Zhong WZ, Zhang XC, Su J, Xie Z, et al. (2017) Potential predictive value of TP53 and *KRAS* mutation status for response to PD-1 blockade immunotherapy in lung adenocarcinoma. Clin Cancer Res 23:3012-3024.

31. Landre T, Justeau G, Assié JB, Chouahnia K, Davoine C, et al. (2022) Anti-PD-(L)1 for *KRAS*-mutant advanced non-small-cell lung cancers: A meta-analysis of randomized-controlled trials. Cancer Immunol Immunother 71:719-726.

32. Wang S, He Z, Wang X, Li H, Liu XS, et al. (2019) Antigen presentation and tumor immunogenicity incancer immunotherapy response prediction. Elife 8:1.

33. Meraviglia-Crivelli D, Zheleva A, Barainka M, Moreno B, Villanueva H, et al. (2022) Therapeutic strategies to enhance tumor antigenicity: Making the tumor detectable by the immune system. Biomedicines 10:1.

34.Ma W, Pham B, Li T (2022) Cancer neoantigens as potential targets for immunotherapy. Clin Exp Metastasis 39:51-60.

35.Gong Z, Yang Y, Zhang J, Guo W (2021) Evaluation of 30 DNA damage response and 6 mismatch repair gene mutations as biomarkers for immunotherapy outcomes across multiple solid tumor types. Cancer Biol Med 18:1080-1091.

36.Peyraud F, Italiano A (2020) Combined PARP inhibition and immune checkpoint therapy in solid tumors. Cancers (Basel) 12:1.

37.Sun W, Zhang Q, Wang R, Li Y, Sun Y, et al. (2021) Targeting DNA damage repair for immune checkpoint inhibition: Mechanisms and potential clinical applications. Front Oncol 11:648687.

38.Shi C, Qin K, Lin A, Jiang A, Cheng Q, et al. (2022) The role of DNA Damage Repair (DDR) system in response to Immune Checkpoint Inhibitor (ICI) therapy. J Exp Clin Cancer Res 41:268.

39.Mouw KW, Goldberg MS, Konstantinopoulos PA, D'Andrea AD (2017) DNA damage and repair biomarkers of immunotherapy response. Cancer Discov 7:675-693.

40.Chen Y, Xu J, Wu X, Yao H, Yan Z, et al. (2021) CD147 regulates antitumor CD8 (+) T-cell responses to facilitate tumor-immune escape. Cell Mol Immunol 18:1995-2009.

41.Alvarez M, Simonetta F, Baker J, Morrison AR, Wenokur AS, et al. (2020) Indirect impact of PD-1/PD-L1 blockade on a murine model of NK cell exhaustion. Front Immunol 11:7.

42.Paul M, Ohashi PS (2020) The roles of CD8 (+) T cell subsets in antitumor immunity. Trends Cell Biol 30:695-704.

43.Wagner AK, Kadri N, Tibbitt C, van de Ven K, Bagawath-Singh S, et al. (2022) PD-1 expression on mouse intratumoral NK cells and its effects on NK cell phenotype. iScience 25:105137.

44.Gomez-Gutierrez JG, Garcia-Garcia A, Hao H, Rao XM, Montes de Oca-Luna R, et al. (2010) Adenovirus-mediated expression of truncated E2F-1 suppresses tumor growth *in vitro* and *in vivo*. Cancer 116:4420-4432.

45.Gao Z, Shi R, Yuan K, Wang Y (2016) Expression and prognostic value of E2F activators in NSCLC and subtypes: A research based on bioinformatics analysis. Tumour Biol 37:14979-14987.

46.Wu SY, Xiao Y, Wei JL, Xu XE, Jin X, et al. (2021) MYC suppresses STING-dependent innate immunity by transcriptionally upregulating DNMT1 in triple-negative breast cancer. J Immunother Cancer 9:1.

47.Kim LC, Cook RS, Chen J (2017) mTORC1 and mTORC2 in cancer and the tumor microenvironment. Oncogene 36:2191-2201.

48. Zeng H, Tong F, Bin Y, Peng L, Gao X, et al. (2022) The predictive value of pak7 mutation for immune checkpoint inhibitors therapy in non-small cell cancer. Front Immunol 13:834142.

49.Thomson AW, Turnquist HR, Raimondi G (2009) Immunoregulatory functions of mTOR inhibition. Nat Rev Immunol 9:324-337.

50.Huang W, Zhang Y, Chen S, Yin H, Liu G, et al. (2022) Personalized immune subtypes based on machine learning predict response to checkpoint blockade in gastric cancer. Brief Bioinform 24:1.