Frontiers in Bioscience Landmark

Front. Biosci. (Landmark Ed) **2022**; 27(3): 103
https://doi.org/10.31083/j.fbl2703103

Original Research

KMT2C is a Potential Biomarker of Anti-PD-1 Treatment Response in Metastatic Melanoma

Kuixia Xie[1], Yonglin Peng[2], Wenying Zhong[1,*], Xinhua Liu[3,*]

[1]Department of Dermatology, The Affiliated Hospital of Hangzhou Normal University, 310015 Hangzhou, Zhejiang, China
[2]Shanghai Center for Systems Biomedicine, Shanghai Jiao Tong University, 200240 Shanghai, China
[3]Department of Biochemistry and Molecular Biology, School of Basic Medical Sciences, Hangzhou Normal University, 311121 Hangzhou, Zhejiang, China
*Correspondence: liuxinhua@tmu.edu.cn (Xinhua Liu); sczjhzzwy@126.com (Wenying Zhong)
Academic Editor: Graham Pawelec
Submitted: 30 December 2021 Revised: 9 February 2022 Accepted: 1 March 2022 Published: 17 March 2022

Abstract

Background: Metastatic melanoma (MM) represents a common malignancy with poor prognosis. Immune checkpoint inhibition (ICI), including PD-1 blockade, has been emerging as the popular therapeutic in MM for its durable treatment effect, but its response rate is still limiting. **Methods**: We comprehensively analyzed the associations between KMT2C somatic mutation and the tumor microenvironment as well as the ICI response of MM patients based on three published cohorts. Gene differential expression analysis between tumor samples with mutated and wild-type KMT2C was performed by DESeq2 package. Functional enrichment analysis was conducted by using clusterProfiler package. Kaplan-Meier was used to perform overall survival probability estimate through survival package and rms package was applied for the construction of nomogram model. **Results**: We report here that KMT2C is a potential biomarker for anti-PD-1 treatment in MM. This biomarker can be used for comprehensively analyzing its association with patients' prognosis, tumor microenvironment and genomic features. Mutations of KMT2C profoundly altered expression of immune- and DNA replication-related genes in MM tumors. MM patients harboring KMT2C mutations showed significantly better overall survival (OS) after treatment with PD-1 monoclonal antibody as compared to wild-type KMT2C. Although KMT2C mutation has no significant influence on immune cell infiltration into MM tumors, the tumor mutation load and neoantigen load are indeed elevated in KMT2C mutated MM samples. This might represent a possible pathway through which KMT2C regulates the response of MM patients to anti-PD-1 treatment. Finally, we constructed a nomogram model by combing the independent prognostic factors, including KMT2C mutation, which could effectively predict the 1-year survival probability of MM patients after anti-PD-1 treatment. **Conclusions**: In conclusion, we report the role of KMT2C in anti-PD-1 treatment response regulation in MM for the first time. This may consequently be helpful for KMT2C personalized application.

Keywords: metastatic melanoma; immune checkpoint; PD-1; KMT2C; tumor microenvironment; neoantigen

1. Introduction

Metastatic melanoma is a common malignancy with increasing incidence worldwide. Although the 5-year survival probability of localized melanoma could achieve 99% after surgical resection of the malignant lesions, the prognosis of metastatic melanoma (MM) is still poor with a 5-year survival probability of only about 20% [1]. Many therapeutics have been proposed for MM, such as adjuvant radio-/chemo-therapy before or after surgery, however, the efficiency has proven to be limited [2,3]. Immunotherapy has long been a therapy of choice for multiple advanced malignancies. This type of treatment works through modulating tumor intrinsic immunity against tumor cells, such as chimeric antigens receptor-T (CAR-T) cell treatment in lymphomas and leukemias. The CAR-T treatment has shown encouraging anti-tumor efficiency [4]. Immune checkpoint blockade (ICB), mainly including anti-PD-1, anti-PD-L1, and anti-CTLA-4, represents the most popular immunotherapy method in the past decade. Some small-molecular inhibitors have been approved for clinical use by US Food and Drug Administration (FDA). For example, pembrolizumab which targets PD-1 and ipilimumab which targets CTLA-4 [5,6]. MM is one of the most sensitive tumors in which ICB treatment and durable efficiency have been obtained in multiple clinical cohorts [7–9]. However, the response of MM patients to ICB is variable and identification of sensitive biomarkers is still urgently needed for the rational therapeutic schedule.

KMT2C, which is also known as MLL3, is a histone methyltransferase which specifically catalyzes the histone H3 lysine K4 mono-methylation at enhancer regions [10,11]. KMT2C has been extensively studied for its role in genome stability regulation partially through the modulation of DNA damage repair [12]. However, controversial conclusions were drawn for its biological functions under various conditions. Larsson *et al*. [13] illustrated the significant association between KMT2C expression, repression and enhanced colorectal cancer cell growth. While

Dawkins *et al*. [14] has found that depletion of KMT2C could profoundly inhibit the proliferation of pancreatic ductal adenocarcinoma cell lines. Chiappetta *et al*. [15] even showed the opposite effect of KMT2C knockdown on the migration capacity comparing primary and metastatic osteosarcoma cell lines. These observations indicate that KMT2C might execute biological functions in a context-dependent manner. Clinically, its mutation or aberrant expression has been widely associated with the prognosis or treatment sensitivity in multiple cancers [16–19]. Our previous study [20] demonstrated the profound influence of KMT2C towards the sensitivity of breast cancer on chemotherapy through the regulation of genome stability. Response to ICB treatment has been closely related to genome instability in multiple cancers, however, the association between KMT2C and ICB response was rarely reported.

We report here for the first time the association between KMT2C mutation and ICB treatment including anti-PD-1 in MM. This might serve as a potential biomarker for personalized therapeutic schedule of MM patients.

2. Material and Methods

2.1 Study Subjects

Three MM cohorts used in this study included 68 MM samples from the Cancer Genome Atlas SKCM cohort (TCGA-SKCM), 144 MM samples from the study of Liu *et al*. [21] (DFCI2019), and 110 MM samples from the study of Allen *et al*. [22] (DFCI2015). Genome-wide mutation profiles of all the three MM cohorts and gene expression profiles of the TCGA-SKCM and DFCI2019 cohorts were accessible and were used for the analysis of this study.

2.2 Differential Expression Analysis

DESeq2 function package [23] of R programming software version4.0.2 was applied to screen differential expression genes (DEGs) in KMT2C mutated (KMT2CMut) MM tumor samples. These were compared to KMT2C wild-type (KMT2CWT) samples in the TCGA-SKCM cohort. Absolute value of log2 (Fold Change) (log2FC) >1 and p-value < 0.05 was used as the significant threshold.

2.3 Gene Set Enrichment Analysis

Functional enrichment analysis of DEGs was conducted by clusterProfiler function package [24] of R programming software with the significant threshold of p-value < 0.05. In addition, Gene Set Enrichment Analysis (GSEA) was also performed in order to identify biological pathways that were significantly repressed and activated by KMT2C mutation with the threshold of FDR <0.05.

2.4 Survival Analysis

Overall survival (OS) probability of MM patients after anti-PD-1 treatment in DFCI2019 cohort was estimated via Kaplan-Meier method by using the survival function package (https://CRAN.R-project.org/package=survival) of R programming software. Score test was applied to determine the significance of OS probability difference among all MM patient groups with the threshold of p-value < 0.05. Multivariate cox regression analysis was used to identify factors that could independently influence the OS probability of MM patients after anti-PD-1 treatment having the threshold of p-value < 0.1.

2.5 Nomogram Model Construction

Nomogram represents a useful means of the prediction of survival probability at specific time points. In this study, we constructed a nomogram model for predicting the 1-, 2-, and 3-year OS probability after anti-PD-1 treatment using the rms function package (https://CRAN.R-project.org/package=rms) of R programming software and included the independent factors in the multivariate cox regression analysis.

2.6 Statistical Analysis

Comparisons of quantitative variables, including tumor mutation burden (TMB), neoantigen load (NAL), between KMT2CMut and KMT2CWT MM samples were conducted using two-sided student t-test with the threshold of p-value < 0.05. Fisher's exact test was used to determine the significance of difference of sample distribution between KMT2CMut and KMT2CWT MM samples. All the statistical analyses of this study were performed in R programming software.

3. Results

3.1 KMT2C is Highly Mutated in MM Patients and Associated with Immune-Related Pathways

KMT2C has been reported to frequently mutate in multiple cancers. In this study, consistent high mutation frequency of KMT2C was observed in the three MM patient cohorts (11 out of 68 MM patients, 16.2% in TCGA-SKCM cohort, 18 out of 144 MM patients, 12.5% in DFCI2019 cohort, 16 out of 110 MM patients, 14.5% in DFCI2015 cohort). In addition to that, based on the TCGA-SKCM cohort, lollipop plot illustrating the distribution of KMT2C mutation sites across its protein functional domains are presented in (Fig. 1A). Lollipop plots of DFCI2019 and DFCI2015 cohorts are provided in **Supplementary Fig. 1**. It was observed that significant enrichment of mutations in the first PHD domain of KMT2C was obtained, which is consistent with the previous report [16].

To explore the functional consequence of KMT2C mutation, we screened the genes that were differentially expressed in KMT2CMut samples compared to KMT2CWT samples in the TCGA-SKCM cohort. The result showed that 1422 significantly down-regulated genes and 317 up-regulated genes were present (Fig. 1B). This is consistent with the transcriptional activation role of KMT2C as a histone methyltransferase. Functional enrichment analysis

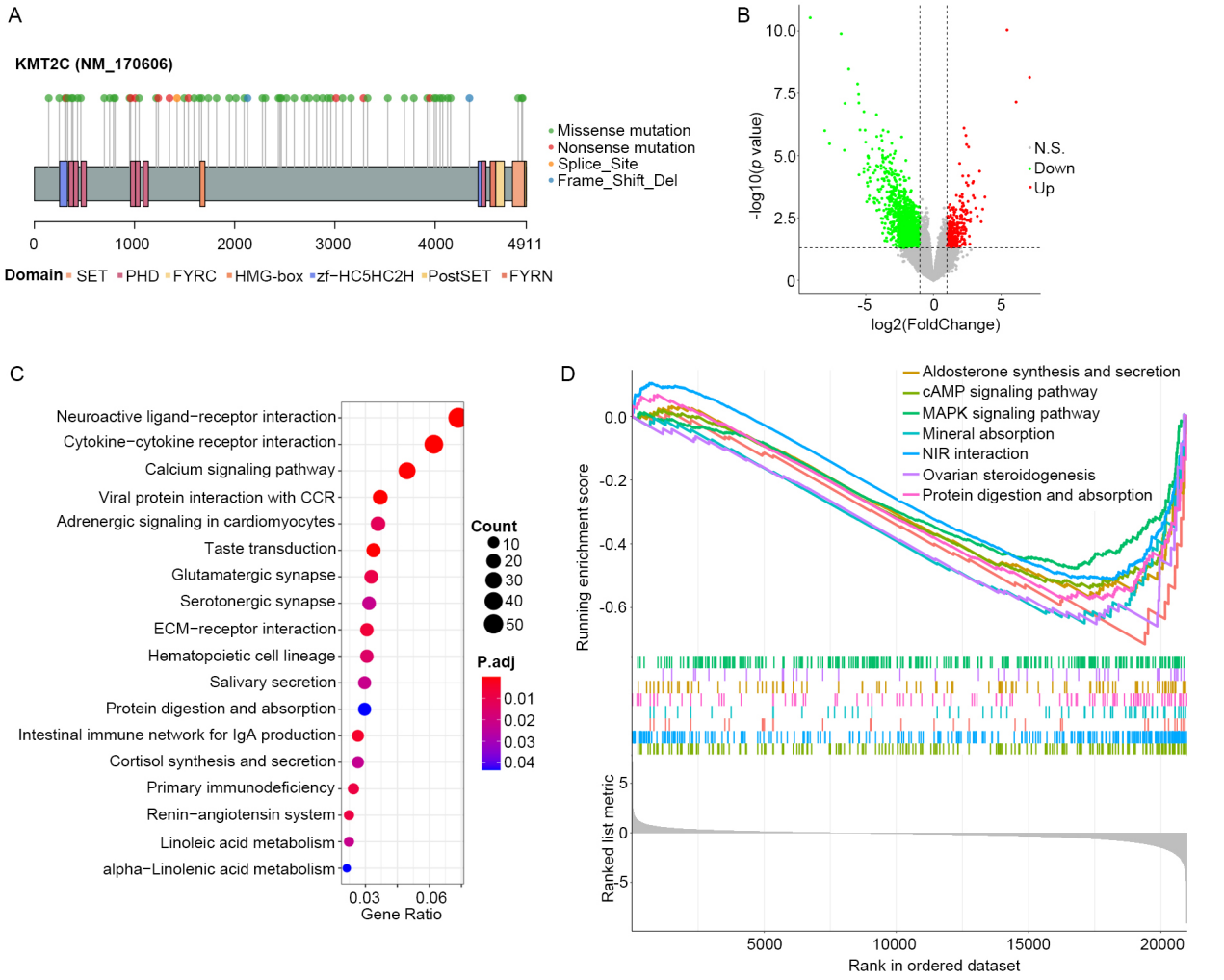

Fig. 1. KMT2C is functionally associated with immune regulation. (A) Lollipop plot illustrating KMT2C mutations in MM samples in the TCGA-SKCM cohort across function domains of KMT2C protein. (B) Volcano plot showing the result of differential expression analysis between KMT2CMut and KMT2CWT MM samples in the TCGA-SKCM cohort. Green and red dots represent significantly down- and up-regulated genes in KMT2CMut compared with KMT2CWT MM samples, and grey dots are nonsignificant genes. Vertical and horizontal dashed line indicate the significant threshold of |log2FC| and *p*-value, respectively. (C) Significantly enriched KEGG pathways of DEGs obtained and visualized through clusterProfiler. (D) The seven KEGG pathways that significantly repressed in KMT2CMut compared with KMT2CWT MM samples in the TCGA-SKCM cohort. NIR, Neuroactive ligand-receptor.

showed a total of 18 significantly enriched KEGG pathways (Fig. 1C) of the 1739 DEGs, including those that were closely related to tumor microenvironment status, such as cytokine-cytokine receptor interaction, ECM-receptor interaction, intestinal immune network for IgA production, and primary immunodeficiency among others. In addition to that, GSEA also identified seven significantly repressed KEGG pathways in KMT2CMut MM samples as shown in (Fig. 1D). These pathways were all cancer-related, e.g., cAMP signaling pathway, MAPK signaling pathway. These results reveal the potential role of KMT2C in cancer and immune regulation.

3.2 KMT2C is an Independent Factor for Anti-PD-1 Response of MM Patients

Investigation of the functional aspect of KMT2C mutations indicated its possible influence on tumor microenvironment, which further prompted us to explore the association between KMT2C mutation and immunotherapy response. Anti-PD-1 monoclonal antibody signifies one of the major immunotherapy methods which has been extensively used in MM research. In this study, we investigated the association between KMT2C mutations and prognosis of MM patients after anti-PD-1 treatment within DFCI2019 MM cohort. This included 144 MM samples that received nivolumab or pembrolizumab, the two most common anti-PD-1 reagents. The result of this study showed that KMT2C mutations are significantly associated with

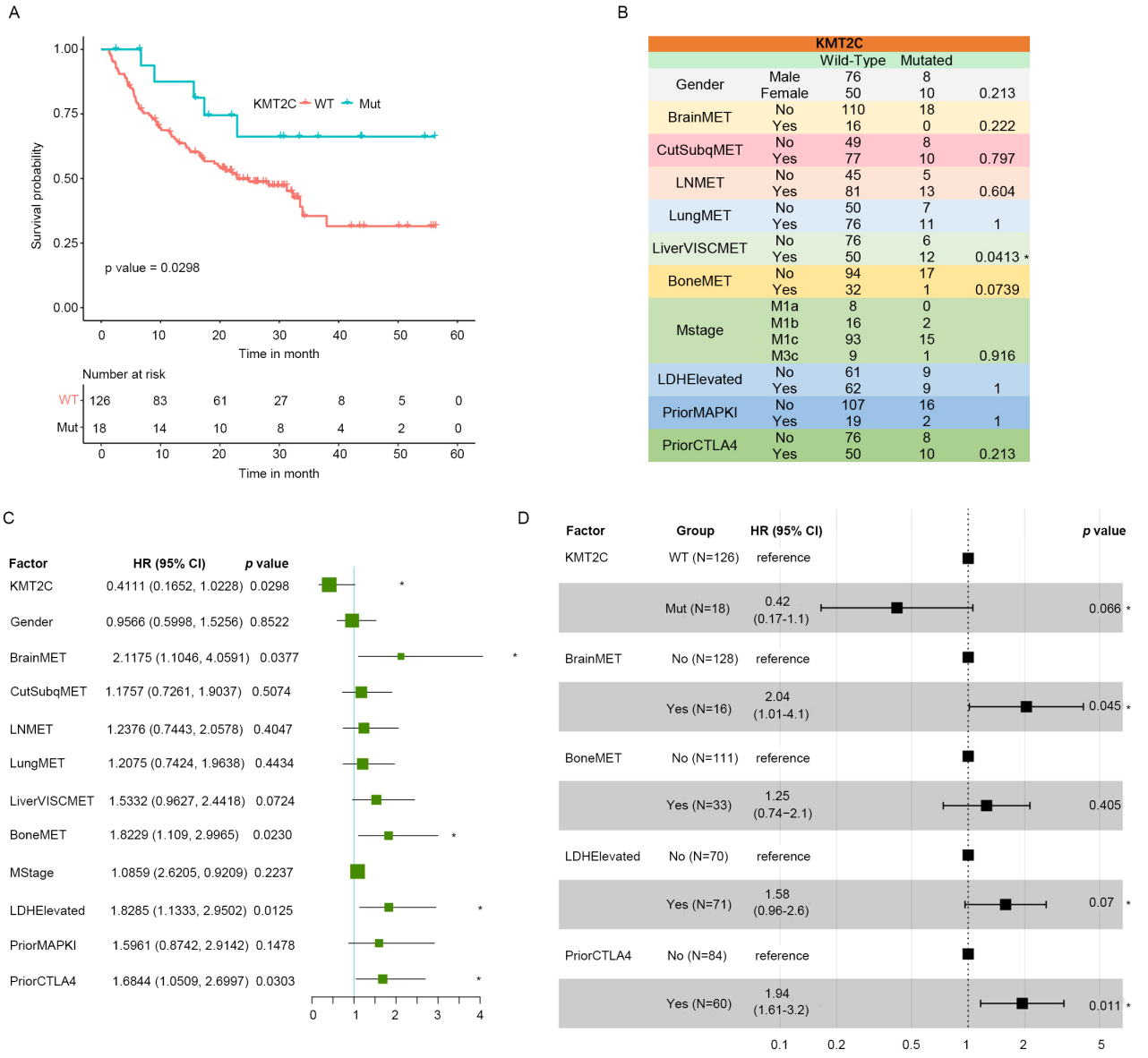

Fig. 2. KMT2C is an independent prognostic factor in anti-PD-1 treated MM samples. (A) Kaplan-Meier plot of MM samples in the DFCI2019 cohort stratified by the KMT2C mutate status. *p*-value was determined by score test using the survival function package. (B) Confounding factor distribution among KMT2CMut and KMT2CWT MM samples in the DFCI2019 cohort. *p*-value was determined by Fisher's exact test. BrainMET, Brain metastasis; CutSubqMET, Subcutaneous metastasis; LNMET, Lymph node metastasis; LungMET, Lung metastasis; LiverVISCMET, Liver metastasis; BoneMET, Bone metastasis; LDHElevated, LDH level; PriorMAPKI, MAPK inhibition treatment status before anti-PD-1 treatment; PriorCTLA4, anti-CTLA4 treatment status before anti-PD-1 treatment. (C) Forest plot of the result of univariate cox regression analysis for the association between KMT2C as well as other confounding factors and OS probability of MM patients after anti-PD-1 treatment in DFCI2019 cohort. * indicates significant association at the threshold of *p*-value < 0.05. (D) Forest plot of the result of multivariate cox regression analysis of the association of OS probability of anti-PD-1 treated MM patients in DFCI2019 cohort with factors that are significant in (C). * indicates significant association at the threshold of *p*-value < 0.1.

higher OS probability after anti-PD-1 treatment when compared to KMT2C wild-type MM samples (Fig. 2A). Some confounding factors might also be associated with the prognosis of MM patients, such as gender, anti-CTLA4 treatment before anti-PD-1 treatment, metastatic sites, etc. To exclude the potential impact of these confounding factors on the association between KMT2C mutation and response of MM patients to anti-PD-1 treatment, we performed Fisher's exact test. This test was performed in order to establish if there was any significant difference in these factors between KMT2CMut and KMT2CWT MM samples. As a result, only liver metastasis showed sig-

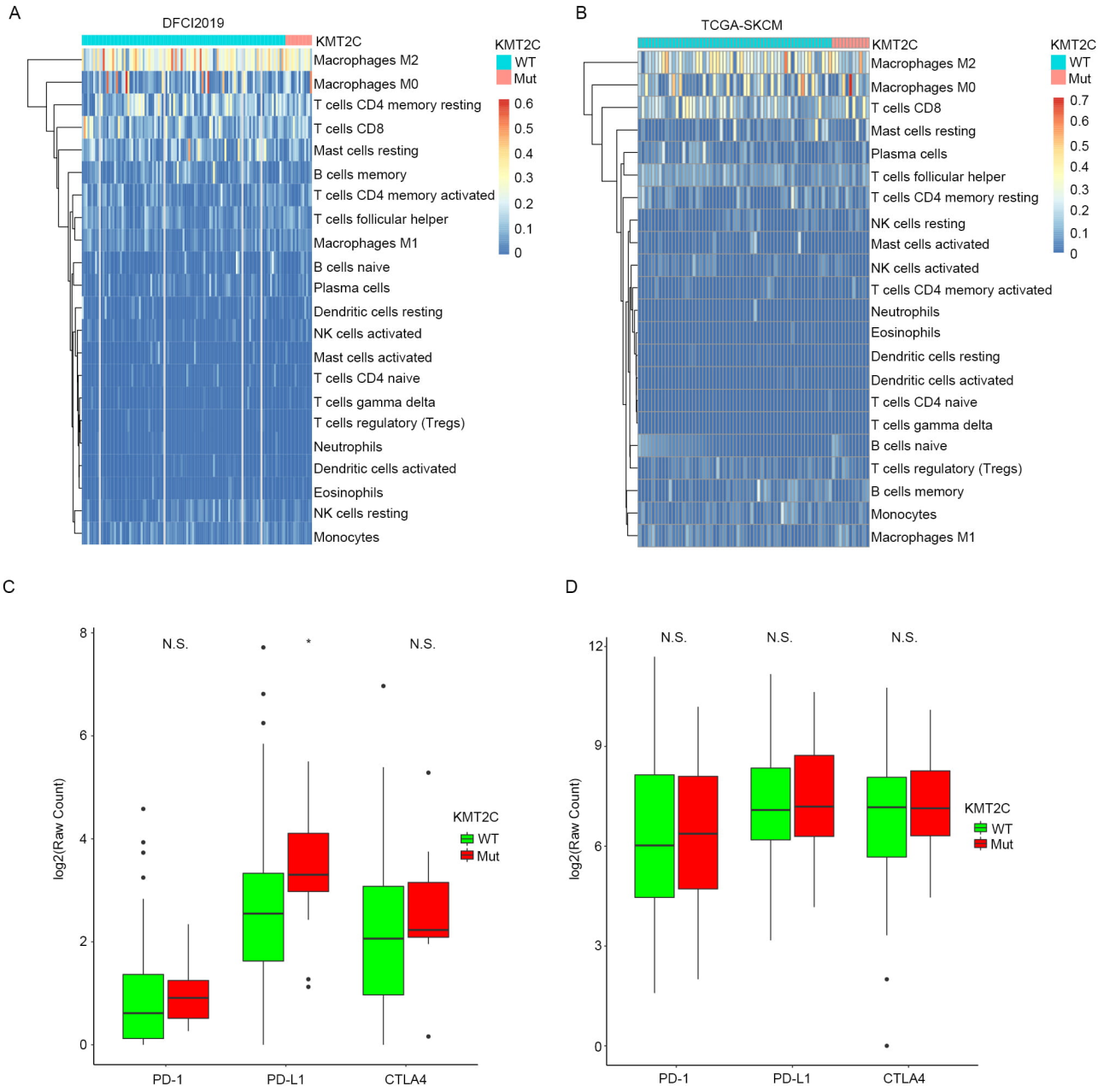

Fig. 3. Association between KMT2C mutation and immune cell infiltration into MM tumor tissue. (A) Heatmap illustrating infiltration level of the 22 immune cells into tumor tissues of MM patients in DFCI2019 cohort stratified by KMT2C mutate status. (B) The same as (A) but in the TCGA-SKCM cohort. (C) Boxplot of the relative mRNA level of three immune checkpoint genes, including PD-1, PD-L1 and CTLA4, in KMT2CMut and KMT2CWT MM samples in DFCI2019 cohort. N.S. indicates not significant and * indicates significant difference at the threshold of p-value < 0.05. (D) The sample as in (A) but in the TCGA-SKCM cohort.

nificant difference between KMT2CMut and KMT2CWT MM samples. In particular, KMT2CMut MM patients contained higher proportion of liver metastasis samples than KMT2CWT MM patients (Fig. 2B). In addition to that, we performed univariate cox regression analysis for all those confounding factors to verify their associations with OS probability of anti-PD-1 treated MM patients. Four factors in addition to KMT2C mutation, including brain metastasis, bone metastasis, lactate dehydrogenase (LDH) level, and anti-CTLA4 treatment status before anti-PD-1 treatment, were found to be potentially associated with OS probability of anti-PD-1 treated MM patients (Fig. 2C). To determine if KMT2C is an independent factor for the response to anti-PD-1 treatment of MM patients, we performed multivariate Cox regression analysis by including all significant factors in the univariate Cox regression analysis. As a result, KMT2C mutation, brain metastasis, LDH level, and anti-CTLA4 status were statistically determined as in-

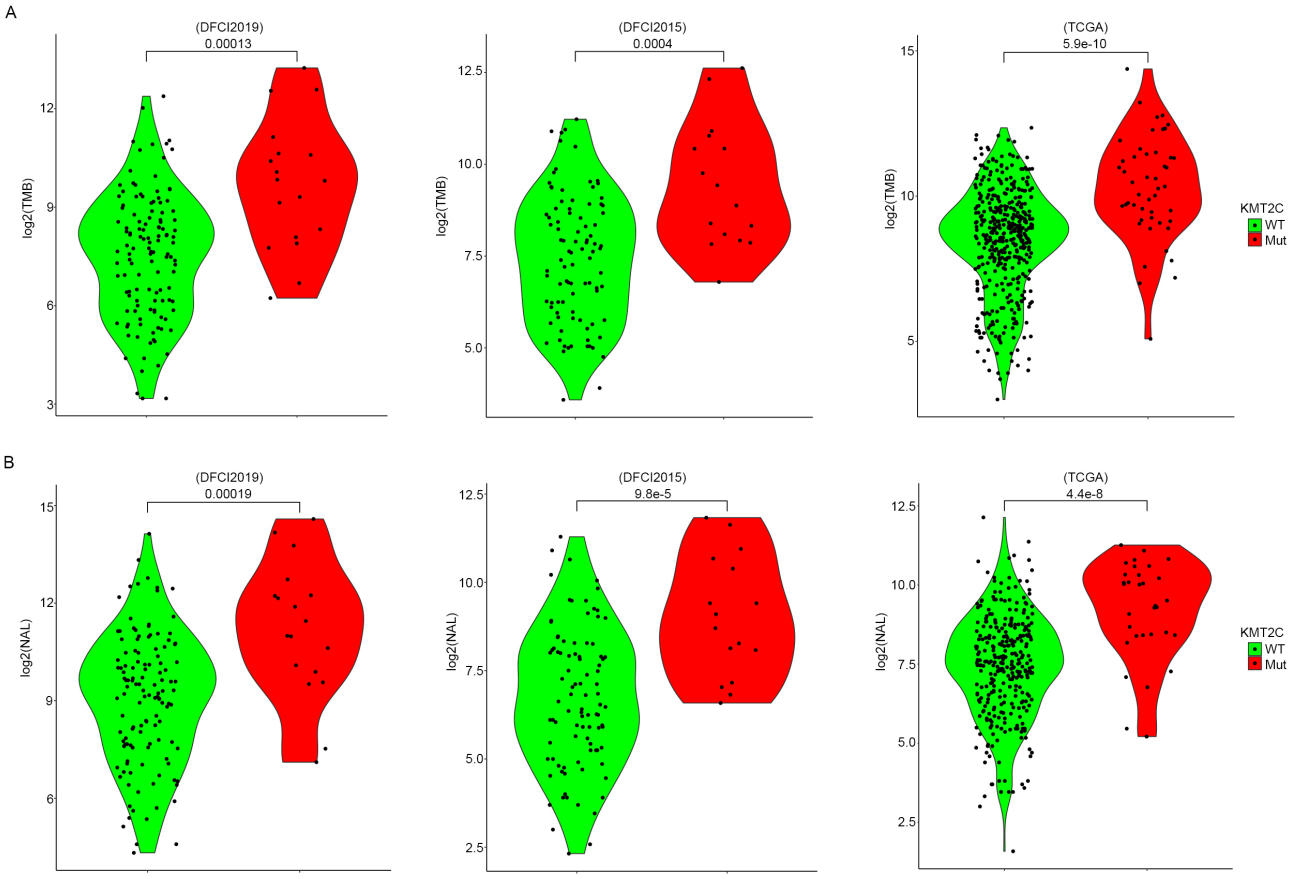

Fig. 4. Influence of KMT2C mutation on TMB and NAL of tumor tissue of MM patients. (A) TMB level (log2-based) of MM samples stratified by KMT2C mutate status in DFCI2019 (left), DFCI2015 (middle), and TCGA-SKCM (right) cohort. The *p*-value was determined by two-sided student *t*-test. (B) NAL level (log2-based) of MM samples stratified by KMT2C mutate status in DFCI2019 (left), DFCI2015 (middle), and TCGA-SKCM (right) cohort. The *p*-value was determined by two-sided student *t*-test.

dependent prognostic factors of anti-PD-1 treated MM patients with the threshold of p-value < 0.1. These results indicate KMT2C might be a reliable independent biomarker in response to anti-PD-1 treatment of MM patients.

3.3 KMT2C Mutation has No Effect on Immune Cell Infiltration into Tumor

Tumor immune cell infiltration has been widely studied for its association with host intrinsic immunity against cancer cells as well as with cancer immunotherapy response. We hypothesized that regulation of immune cell infiltration into tumor mass might be a potential route through which KMT2C mutation influences the response of MM patients to anti-PD-1 treatment. To test this premise, we obtained the infiltration ratio of 22 immune cells of MM patients in TCGA-SKCM and DFCI2019 cohorts from the study of Charoentong *et al.* [25] and Liu *et al.* [21], respectively. The infiltration levels of the 22 immune cells in the tumor samples of MM patients are shown in the form of heat map in Fig. 3A,B. Macrophage M2, an immune-suppressed cell, showed significantly higher infiltration level than other cell types in MM tumors in both TCGA-SKCM and DFCI2019 MM patient cohorts. Immune activated cell macrophage M1 had relatively low infiltration level. Moreover, cytotoxic T cells, i.e., CD8 T cells, showed a modest infiltration level, which indicated that MM patients might intrinsically function against cancer cells if provided an appropriate tumor microenvironment. It was also observed that none of the 22 immune cells showed significant infiltration difference between KMT2CMut and KMT2CWT MM samples. In addition to that, we further tested if there were any significant differences in common immune checkpoint gene expression, including PD-1, PD-L1 and CTLA4, between KMT2CMut and KMT2CWT MM samples in the TCGA-SKCM and DCFI2019 cohorts. As a result, no gene except PD-L1 in the DFCI2019 cohort (KMT2CWT *versus* KMT2CMut: 2.50 *versus* 3.38, p-value = 0.029) showed significant difference between KMT2CMut and KMT2CWT MM samples in both cohorts (Fig. 3C,D). Those results indicate that KMT2C might influence anti-PD-1 treatment response through other pathways but not immune cell infiltration and checkpoint gene expression.

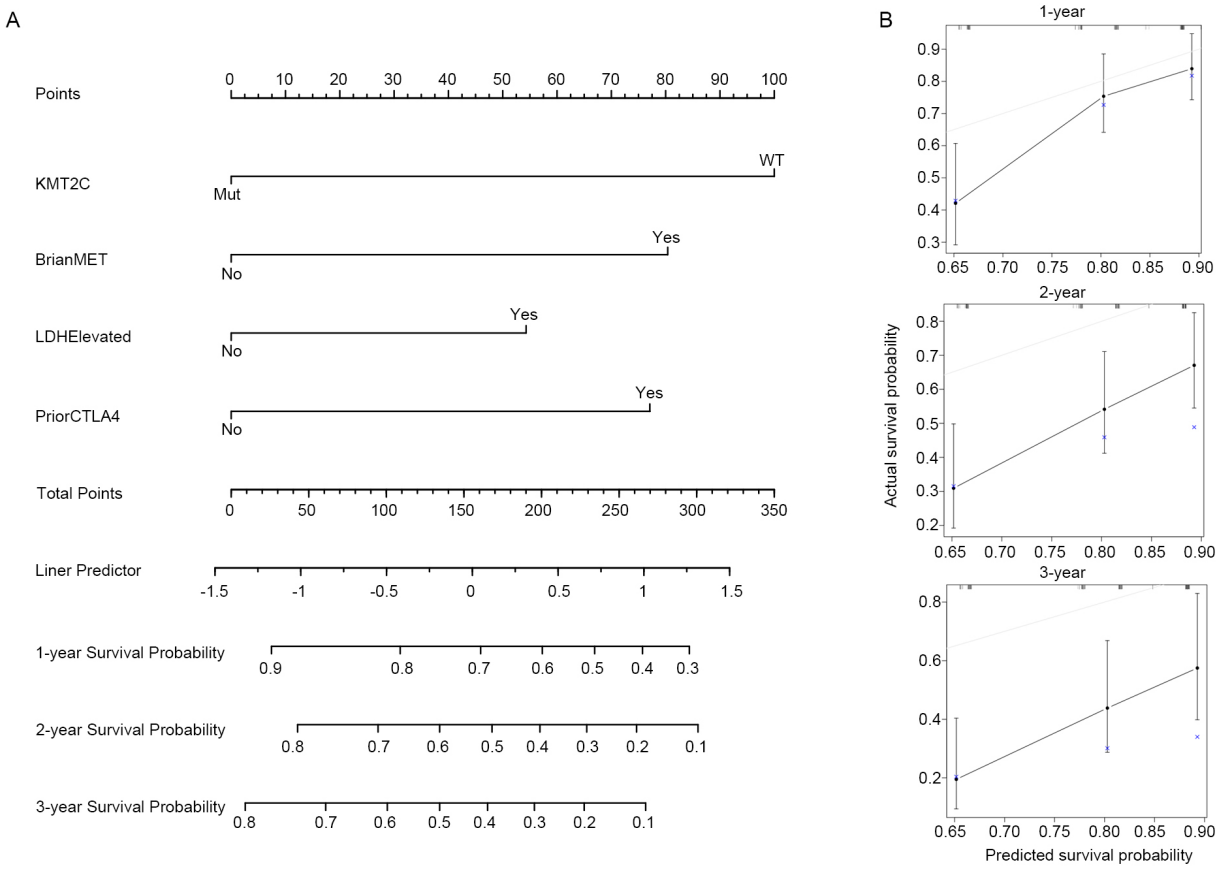

Fig. 5. Nomogram for predicting the prognosis of anti-PD-1 treated MM patients. (A) Nomogram for the 1-, 2-, and 3-year OS probability prediction of MM patients after anti-PD-1 treatment in the DFCI2019 cohort by including the significant factors in multivariate cox regression analysis. (B) Calibration curves for estimating the performance of the nomogram model in predicting the 1-year (top), 2-year (middle), and 3-year (bottom) OS probability of anti-PD-1 treated MM patients in DFCI2019 cohort. X-axis and Y-axis represents the predicted and actual OS probability at the specific time point, respectively.

3.4 KMT2C Mutation is a Genome Mutation Stimulus Associated with Neoantigen Production in MM Tumors

Tumor mutation burden (TMB) represents a widely used prognosis marker in many cancers and has been clinically used for estimating the response of targeted therapy or immunotherapy. Several factors have been closely linked to the TMB level through different pathways, including perturbation in the DNA replication process, DNA damage repair, etc. It has been previously reported that KMT2C is involved in regulating DNA replication as well as DNA damage repair pathways [26], such as homologous recombination. Based on these observations we investigated the association between KMT2C mutation and TMB in MM patients. TMB in this study was defined as the total number of nonsense and missense mutations per Mb genome. The result indicated that KMT2CMut MM samples show significantly higher TMB compared to KMT2CWT MM samples in all the three MM patient cohorts (Fig. 4A). Neoantigen is a type of antigen presented in the tumor cell surface and is highly individual-specific. Presence of neoantigen effectively increases the identification probability of tumor cells by cytotoxicity T cell and is closely associated with a better prognosis of multiple cancers [27–29]. In addition, the occurrence of mutation also endorsed that it contributes to the formation of neoantigen. TMB has been reported to be positively correlated with neoantigen load (NAL) in lots of studies, including our current study (**Supplementary Fig. 2**). Given these aspects, we further compared the NAL in the three MM patient cohorts between KMT2CMut and KMT2CWT samples. As expected, the NAL in KMT2CMut samples proved to be significantly higher than those KMT2CWT samples in all three cohorts (Fig. 4B). This indicates that the enhancement of NAL should be a potential path with which KMT2C mutation sensitizes the response of MM patients to anti-PD-1 treatment.

3.5 A Nomogram Predicting the Short-Term OS Probability of MM Patients after Anti-PD-1 Treatment

Nomogram is widely used for predicting disease prognosis that consists of multiple relevant factors. In this study, we constructed a nomogram that predicted the 1-, 2-, and

3-year OS probability of MM patients after anti-PD-1 treatment in DFCI2019 cohort. This included the independent prognostic factors in the multivariate cox regression analysis, i.e., KMT2C mutation, brain metastasis, LDH level, and anti-CTLA4 status (Fig. 5A). It was found that KMT2C mutation, brain metastasis-free, low LDH level, and naïve anti-CTLA4 before anti-PD-1 treatment, were validated to be associated with higher 1-, 2-, and 3-year OS probability of MM patients after anti-PD-1 treatment. To evaluate the performance of the combined nomogram in predicting the OS probability of anti-PD-1 treated MM patients at different time points, the calibration curves for 1-, 2-, and 3-year OS probability were plotted to estimate the deviation between the actual and nomogram predicted OS probability. As a result, although the deviation was relatively large between the actual and nomogram predicted anti-PD-1 treated MM patient OS probability at 2- and 3-year, it was indeed very small at 1-year (Fig. 5B). Based on this study the nomogram might be useful in predicting the short-term OS probability of MM patients after anti-PD-1 treatment.

4. Discussion

Immunotherapy in the last decade has been revolutionarily developed and applied in multiple cancers [30–32]. MM is one of the most common scenarios that immunotherapy, particularly ICB is used for its intrinsic high TMB which underlies the highly activated tumor immunity [33–35]. Durable effects of ICB in MM have been widely obtained, but the low response rate largely impedes its extensive application. Identifying response biomarkers for ICB is currently in urgent need to accelerate the robust and rapid development of ICB application in cancer [36]. Here, we report KMT2C as a potential anti-PD-1 treatment marker in MM patients.

KMT2C is a well-known epigenetic regulator that plays important role in transcriptional regulation, specifically activation, by loosening chromatin structure through its catalytic role in mono-methylation of histone H3 lysine K4 (H3K4) [37]. Epigenetic regulation plays fundamental roles in lots of biological and clinical aspects [38,39]. KMT2C was previously shown to contribute to the genomic stability and its mutation leads to the obvious TMB elevation in multiple cancers [20,40]. In this study, a consistent positive association between KMT2C mutation and higher TMB in MM patients was observed, which also might result in the increase of NAL level. Neoantigen, which is produced by the mutation in exon part of the gene, is unlike the common antigen, such as carcinoembryonic antigen, which is highly specific to each individual and widely used for the design of personalized cancer vaccine [41–43]. In addition, high NAL would profoundly enhance the identification of cancer cells by cytotoxicity T cells and induce immune response for cancer cell removal, which is closely associated with better clinical manifestation, such as slow cancer progression and prolonged overall survival [27,44]. Here we propose that KMT2C mutation contributes to a better anti-PD-1 response of MM patients which might be tightly related to its correlation with the elevation of TMB and later the NAL level. The role of KMT2C in immunotherapy response in other cancers was sporadically reported [45,46], but the understanding of underlying mechanisms is very poorly understood and further studies are still needed.

5. Conclusions

In conclusion, we report here the potential role of KMT2C in the regulation of response of MM patients to anti-PD-1 treatment for the first time. This should be helpful for future studies regarding the sensitivity and extent of the clinical use of ICB treatment.

Author Contributions

KX analyzed the data and wrote the manuscript. YP analyzed the data. WZ and XL proposed the study and revised the writing.

Ethics Approval and Consent to Participate

Not applicable.

Acknowledgment

Not applicable.

Funding

This research received no external funding.

Conflict of Interest

The authors declare no conflict of interest.

Supplementary Material

Supplementary material associated with this article can be found, in the online version, at https://www.imrpress.com/journal/FBL/27/3/10.31083/j.fbl2703103.

References

[1] Leonardi GC, Candido S, Falzone L, Spandidos DA, Libra M. Cutaneous melanoma and the immunotherapy revolution (Review). International Journal of Oncology. 2020; 57: 609–618.

[2] Wada-Ohno M, Ito T, Furue M. Adjuvant Therapy for Melanoma. Current Treatment Options in Oncology. 2019; 20: 1–14.

[3] Testori AAE, Blankenstein SA, van Akkooi ACJ. Surgery for Metastatic Melanoma: An Evolving Concept. Current Oncology Reports. 2019; 21: 98.

[4] Ramos CA, Heslop HE, Brenner MK. CAR-T Cell Therapy for Lymphoma. Annual Review of Medicine. 2016; 67: 165–183.

[5] Bagchi S, Yuan R, Engleman EG. Immune Checkpoint Inhibitors for the Treatment of Cancer: Clinical Impact and Mechanisms of Response and Resistance. Annual Review of Pathology: Mechanisms of Disease. 2021; 16: 223–249.

[6] Dolladille C, Ederhy S, Sassier M, Cautela J, Thuny F, Cohen AA, *et al.* Immune Checkpoint Inhibitor Rechallenge After Immune-Related Adverse Events in Patients with Cancer. JAMA Oncology. 2020; 6: 865–871.

[7] Herrscher H, Robert C. Immune checkpoint inhibitors in melanoma in the metastatic, neoadjuvant, and adjuvant setting. Current Opinion in Oncology. 2020; 32: 106–113.
[8] Queirolo P, Boutros A, Tanda E, Spagnolo F, Quaglino P. Immune-checkpoint inhibitors for the treatment of metastatic melanoma: a model of cancer immunotherapy. Seminars in Cancer Biology. 2019; 59: 290–297.
[9] Barrios DM, Do MH, Phillips GS, Postow MA, Akaike T, Nghiem P, *et al*. Immune checkpoint inhibitors to treat cutaneous malignancies. Journal of the American Academy of Dermatology. 2020; 83: 1239–1253.
[10] Xue H, Yao T, Cao M, Zhu G, Li Y, Yuan G, *et al*. Structural basis of nucleosome recognition and modification by MLL methyltransferases. Nature. 2019; 573: 445–449.
[11] Li Y, Han J, Zhang Y, Cao F, Liu Z, Li S, *et al*. Structural basis for activity regulation of MLL family methyltransferases. Nature. 2016; 530: 447–452.
[12] Je EM, Lee SH, Yoo NJ, Lee SH. Mutational and expressional analysis of MLL genes in gastric and colorectal cancers with microsatellite instability. Neoplasma. 2013; 60: 188–195.
[13] Larsson C, Cordeddu L, Siggens L, Pandzic T, Kundu S, He L, *et al*. Restoration of KMT2C/MLL3 in human colorectal cancer cells reinforces genome-wide H3K4me1 profiles and influences cell growth and gene expression. Clinical Epigenetics. 2020; 12: 74.
[14] Dawkins JBN, Wang J, Maniati E, Heward JA, Koniali L, Kocher HM, *et al*. Reduced Expression of Histone Methyltransferases KMT2C and KMT2D Correlates with Improved Outcome in Pancreatic Ductal Adenocarcinoma. Cancer Research. 2016; 76: 4861–4871.
[15] Chiappetta C, Carletti R, Della Rocca C, Di Cristofano C. KMT2C modulates migration and invasion processes in osteosarcoma cell lines. Pathology - Research and Practice. 2019; 215: 152534.
[16] Fagan RJ, Dingwall AK. COMPASS Ascending: Emerging clues regarding the roles of MLL3/KMT2C and MLL2/KMT2D proteins in cancer. Cancer Letters. 2019; 458: 56–65.
[17] Wang L, Shilatifard A. UTX Mutations in Human Cancer. Cancer Cell. 2019; 35: 168–176.
[18] Huang R, Zhu L, Zhang Y. XIST lost induces ovarian cancer stem cells to acquire taxol resistance via a KMT2C-dependent way. Cancer Cell International. 2020; 20: 436.
[19] Chang A, Liu L, Ashby JM, Wu D, Chen Y, O'Neill SS, *et al*. Recruitment of KMT2C/MLL3 to DNA Damage Sites Mediates DNA Damage Responses and Regulates PARP Inhibitor Sensitivity in Cancer. Cancer Research. 2021; 81: 3358–3373.
[20] Liu X, Qiu R, Xu M, Meng M, Zhao S, Ji J, *et al*. KMT2C is a potential biomarker of prognosis and chemotherapy sensitivity in breast cancer. Breast Cancer Research and Treatment. 2021; 189: 347–361.
[21] Liu D, Schilling B, Liu D, Sucker A, Livingstone E, Jerby-Arnon L, *et al*. Integrative molecular and clinical modeling of clinical outcomes to PD1 blockade in patients with metastatic melanoma. Nature Medicine. 2019; 25: 1916–1927.
[22] Van Allen EM, Miao D, Schilling B, Shukla SA, Blank C, Zimmer L, *et al*. Genomic correlates of response to CTLA-4 blockade in metastatic melanoma. Science. 2015; 350: 207–211.
[23] Love MI, Huber W, Anders S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biology. 2014; 15: 550.
[24] Yu G, Wang L, Han Y, He Q. ClusterProfiler: An R package for comparing biological themes among gene clusters. Omics. 2012; 16: 284–287.
[25] Charoentong P, Finotello F, Angelova M, Mayer C, Efremova M, Rieder D, *et al*. Pan-cancer Immunogenomic Analyses Reveal Genotype-Immunophenotype Relationships and Predictors of Response to Checkpoint Blockade. Cell Reports. 2017; 18: 248–262.
[26] Lu F, Wu X, Yin F, Chia-Fang Lee C, Yu M, Mihaylov IS, *et al*. Regulation of DNA replication and chromosomal polyploidy by the MLL-WDR5-RBBP5 methyltransferases. Biology Open. 2016; 5: 1449–1460.
[27] Perumal D, Imai N, Laganà A, Finnigan J, Melnekoff D, Leshchenko VV, *et al*. Mutation-derived Neoantigen-specific T-cell Responses in Multiple Myeloma. Clinical Cancer Research. 2020; 26: 450–464.
[28] Wang Z, Liu W, Chen C, Yang X, Luo Y, Zhang B. Low mutation and neoantigen burden and fewer effector tumor infiltrating lymphocytes correlate with breast cancer metastasization to lymph nodes. Scientific Reports. 2019; 9: 253.
[29] Chen H, Yang G, Xiao J, Zheng L, You L, Zhang T. Neoantigen-based immunotherapy in pancreatic ductal adenocarcinoma (PDAC). Cancer Letters. 2020; 490: 12–19.
[30] Li B, Chan HL, Chen P. Immune Checkpoint Inhibitors: Basics and Challenges. Current Medicinal Chemistry. 2019; 26: 3009–3025.
[31] Sanmamed MF, Chen L. A Paradigm Shift in Cancer Immunotherapy: From Enhancement to Normalization. Cell. 2018; 175: 313–326.
[32] Bergman PJ. Cancer Immunotherapies. The Veterinary Clinics of North America. Small Animal Practice. 2019; 49: 881–902.
[33] Ralli M, Botticelli A, Visconti IC, Angeletti D, Fiore M, Marchetti P, *et al*. Immunotherapy in the Treatment of Metastatic Melanoma: Current Knowledge and Future Directions. Journal of Immunology Research. 2020; 2020: 9235638.
[34] Koppolu V, Rekha Vasigala VK. Checkpoint immunotherapy by nivolumab for treatment of metastatic melanoma. Journal of Cancer Research and Therapeutics. 2018; 14: 1167–1175.
[35] Herzberg B, Fisher DE. Metastatic melanoma and immunotherapy. Clinical Immunology. 2016; 172: 105–110.
[36] LoRusso PM, Schalper K, Sosman J. Targeted therapy and immunotherapy: Emerging biomarkers in metastatic melanoma. Pigment Cell and Melanoma Research. 2020; 33: 390–402.
[37] Liu Y, Qin S, Chen T, Lei M, Dhar SS, Ho JC, *et al*. Structural insights into trans-histone regulation of H3K4 methylation by unique histone H4 binding of MLL3/4. Nature Communications. 2019; 10: 36.
[38] Hogg SJ, Beavis PA, Dawson MA, Johnstone RW. Targeting the epigenetic regulation of antitumour immunity. Nature Reviews Drug Discovery. 2020; 19: 776–800.
[39] Zhang L, Lu Q, Chang C. Epigenetics in Health and Disease. Advances in Experimental Medicine and Biology. 2020; 1253: 3–55.
[40] Feng F, Wu X, Shi X, Gao Q, Wu Y, Yu Y, *et al*. Comprehensive analysis of genomic alterations of Chinese hilar cholangiocarcinoma patients. International Journal of Clinical Oncology. 2021; 26: 717–727.
[41] Ott PA, Hu Z, Keskin DB, Shukla SA, Sun J, Bozym DJ, *et al*. An immunogenic personal neoantigen vaccine for patients with melanoma. Nature. 2017; 547: 217–221.
[42] Peng M, Mo Y, Wang Y, Wu P, Zhang Y, Xiong F, *et al*. Neoantigen vaccine: an emerging tumor immunotherapy. Molecular Cancer. 2019; 18: 128.
[43] Ding Z, Li Q, Zhang R, Xie L, Shu Y, Gao S, *et al*. Personalized neoantigen pulsed dendritic cell vaccine for advanced lung cancer. Signal Transduction and Targeted Therapy. 2021; 6: 26.
[44] Germano G, Lamba S, Rospo G, Barault L, Magri A, Maione F, *et al.* Inactivation of DNA repair triggers neoantigen generation and impairs tumour growth. Nature. 2017; 552: 116–120.
[45] Shi Y, Lei Y, Liu L, Zhang S, Wang W, Zhao J, *et al*. Integration of comprehensive genomic profiling, tumor mutational burden, and PD-L1 expression to identify novel biomarkers of im-

munotherapy in non-small cell lung cancer. Cancer Medicine. 2021; 10: 2216–2231.

[46] Bai X, Wu DH, Ma SC, Wang J, Tang XR, Kang S, *et al.* Development and validation of a genomic mutation signature to predict response to PD-1 inhibitors in non-squamous NSCLC: a multicohort study. Journal for ImmunoTherapy of Cancer. 2020; 8: e000381.