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Genomic landscape, immune characteristics and a prognostic mutation signature of cervical cancer in China

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Abstract

Purpose: This study aimed to analyze genomic alteration profiles and immune characteristics of a cohort of Chinese cervical cancer patients to understand why certain patients benefited from molecular targeted therapies, immunotherapy and their prognostic significance.

Methods: PD-L1 expression, and clinicopathological information from 98 cervical cancer samples. Differences in PD-L1 expression and gene mutations between squamous cell carcinoma (SCC) and adenocarcinoma (AC) were analyzed by chi-square test or Fisher's exact test. Differences in gene mutations between our cohort and the Cancer Genome Atlas (TCGA) cohort were tested by Fisher's exact test. Logistic regression was used to analyze factors influencing TMB-high.

Results: Positive PD-L1 expression was significantly higher in cervical SCC than in cervical AC (87% vs. 39%, $p < 0.001$). Frequently mutated genes in cervical cancer included *PIK3CA, KMT2D*, and *KMT2C* genes, among others. *PIK3CA* gene mutation rates were significantly higher in SCC than in AC ($p = 0.004$). *TERT* gene mutation rate was significantly higher in our cohort than TCGA cohort (12% vs. 1%, $p < 0.001$). The independent predictors of TMB-high were *KMT2C* and *LRP1B* gene mutations ($p < 0.05$). we also found the PTEN mutations were associated with worse survival (median PFS, 12.16 vs. 21.75 months, $p = 0.0024$)

Conclusion: Cervical SCC and AC have different molecular profiling and immune characteristics, suggesting that targeted treatments for SCC and AC patients may improve clinical outcomes. *KMT2C* and *LRP1B* gene mutations are independent predictors of TMB-high status in cervical cancer. We have also proposed the prognostic value of PTEN mutations.

Background

Cervical cancer is the fourth most common cancer and has a high mortality rate among women worldwide. In China, cervical carcinoma is the second most common malignancies among women [1, 2]. Early cervical cancer can be effectively treated by surgery, while concurrent chemoradiation is the first choice for locally advanced cervical cancer. However, 20%–30% of cervical cancer patients have recurrence or distant metastasis after first-line treatment, and the treatment strategies for these patients are limited. Therefore, local recurrence and distant metastasis are the major causes of death in advanced cervical carcinoma.

In recent years, molecularly targeted therapies have been increasingly applied to various malignancies. The comprehensive genetic and molecular characteristics of malignant cervical tumour were released in The Cancer Genome Atlas Research Network [3]. Nevertheless, in China, only a few studies with small sample sizes or sequencing of a small panel of genes have evaluated gene mutations in cervical cancer. Therefore, it is requisite to establish a larger genetic profile of the Chinese population through next-generation sequencing (NGS).

Immunotherapy is an emerging treatment of cervical cancer. However, the KEYNOTE-158 trial showed that the overall response rate of cervical cancer patients treated with immunotherapy was only 12.2% [4]. Malignant tumour patients with positive PD-L1 expression and high tumor mutational burden (TMB) were considered as a patient population that may achieve sustained benefit from pembrolizumab [5]. Therefore, it is critical to distinguish the cervical cancer patients who can benefit from immune checkpoint inhibitor. However, only a few studies with small samples have reported PD-L1 expression and TMB in Chinese populations with cervical cancer [6, 7].

Squamous cell carcinoma (SCC), and adenocarcinoma (AC), are the prime pathological classifications of cervical carcinoma. Some studies have shown that AC has a higher rate of lymph node involvement and distant metastasis compared with SCC, and the prognosis of AC is poor [8-10]. It is reported in a study subgroup analysis that SCC patients have better response to immunotherapy than AC ones [11]. Only a few studies have investigated whether SCC and AC have different molecular profiles among Chinese patients.

The incidence and spectrum of somatic mutations in carcinoma of cervix were analyzed in the current study. We compared the differences in the molecular profiles, PD-L1 expression, and TMB between cervical SCC and AC in a Chinese population. These findings may help guide the targeted therapy of cervical cancer and lead to the identification of new markers that can predict the therapeutic effect of immune checkpoint inhibitors in cervical cancer patients by integrating the expression of PD-L1, genomic variation and TMB.

Methods

Samples and clinical data

We collected 98 patients with cervical cancer in Fujian Cancer Hospital. Clinical data were extracted from electronic medical records. Samples including tumor tissues as well as matched neighbouring normal tissues or peripheral blood were collected.

Immunohistochemistry

Immunohistochemical analysis of PD-L1 expression was performed on 4–5 μm formalin-fixed and paraffin-embedded sections using anti-human PD-L1 antibody (Dako 22C3) according to the manufacturer's recommendations. The combined positive score (CPS) was used to measure PD-L1 protein expression. It was defined that the number of PD-L1-stained cells divided by the whole quantity of surviving tumor cells, and then multiplied by 100. Based on previous studies [11], we set two cut-off values for CPS for PD-L1 expression: 1 and 10. PD- L1 positivity was defined as CPS ≥1.

Genome alignment and variant calling

After removing sequencing reads with low quality and adapter bases using fastp (https://github.com/OpenGene/fastp), clean reads were aligned to the human genome (National Center

for Biotechnology Information build 37, hg19) using Burrows–Wheeler Aligner (https://github.com/lh3/bwa). Sorted BAM files were created using sambamba (https://github.com/biod/sambamba). The duplicate reads were marked using samblaster (https://github.com/GregoryFaust/samblaster). Single nucleotide variants (SNVs) and small insertions and deletions (indels) were called and identified using Varscan2 (http://varscan.sourceforge.net). False positive mutations were removed using in-house filter-tools and IGV (http://www.igv.org) double-checked manually with variant allele frequency ≥1%.

Functional annotation

We used annovar (https://annovar.openbioinformatics.org/en/latest/) to annotate the variant call format. Consensus Coding Sequence and RefSeq were used to determine amino acid variations. The annotation content contained variant positions, variant types, and other information. The dbSNP (http://www.bioinfo.org.cn/relative/dbSNP%20Home%20Page.htm), COSMICV91 (https://cancer.sanger.ac.uk/cosmic), 1000 genomes project (https://www.internationalgenome.org), and Exome Aggregation Consortium (http://exac.broadinstitute.org) databases were also used to obtain the population frequencies and other information for the mutations.

Open-source dataset acquisition and pre-processing

The MAF file and clinical information of cervical cancer was obtained from TCGA database (https://portal.gdc.cancer.gov). To remove the inter-batch effect (TCGA uses whole exome sequencing (WES) sequencing with middle depth, while this project used Novogene PM2.0 panel with high depth), we extracted mutations of pm2.0-covered genes in TCGA data using Python 2.7 (Supplementary TCGA_PM2 data).

Visualization and data statistics

We used R package maftools (http://bioconductor.org/packages/release/bioc/vignettes/maftools/inst/doc/maftools.html) for statistical analysis and visualization, including landscape, statistical testing, and other analyses.

Calculation of TMB

The TMB of cervical cancer patients was calculated as the ratio of the total amount of non-synonymous mutations and the total coding size of the panel (which was 1.2 Mb for the Novogene PM2.0 483 gene panel). The mutation count included non-synonymous SNVs and indels detected within the coding region, with the exclusion of driver gene events (in *EGFR, MET, BRAF, PIK3CA, NF1, KRAS, TP53, NOTCH1, NOTCH2, NOTCH3*, and *NOTCH4* genes). Only mutations with an allelic fraction of ≥ 2.5% for SNVs and 1.3% for indels were included in the mutation count. TMB was divided into high or low with the top quartile as the cutoff value 5.19 muts/MB.

Statistical analysis

Statistical analyses were conducted using GraphPad PRISM (version 8.0.2) and SPSS (version 25.0). Mutation information analysis was performed using R software (version 4.0.1). The differences in PD-L1 expression and gene mutations between SCC and AC were analyzed by the chi-square test or Fisher's exact test. Differences in mutations between our cohort and TCGA cohort was tested by Fisher's exact test. The relationship between gene mutations and TMB was analyzed by the Mann–Whitney U test. Influence factors of TMB-high were analyzed by Logistic regression. Progress free survival (PFS) were plotted by Kaplan-Meier curves, the difference in the survival between two groups was analyzed by the log-rank test. Univariate and multivariate Cox regression analyses were conducted to test whether the prognostic ability of PTEN mutations was an independent prognostic factor. For all analyses, clinical stages were categorized as stages I-II or III-IV. Histological types were categorized as SCC or non-SCC; age was a continuous variable. A p-value < 0.05 was considered significant.

Results

The clinical characteristics and pathological data

The clinical characteristics and pathological data for 98 patients are summarized in Table S1. The median age was 50.5 years (range from 34 to 73). Of 98 patients, 67 patients (68.4%) were Squamous cell carcinoma (SCC), 26 patients (26.5%) were Adenocarcinoma (AC), and 5 patients (5.1%) were Adenosquamous carcinoma (ASC). At initial diagnosis, there were patients 16 (16.3%) at stage I, 34 (34.7%) at stage II, 31 (31.6%) at stage III, and 11(11.2%) at stage IV. PD-L1 positivity was found in 54 (72.0%) of 75 patients. PD-L1 expression status was unknown in 23 patients.

Landscapes of frequently mutated genes in our cohort

We performed mutation spectrum analysis in 98 cervical cancer samples, including 26 AC cases, 67 SCC cases, and 5 ASC cases. Validated gene mutations were detected in 91 of the 98 tumors (93%) (Supplementary mutation data). The top 20 most frequently mutated genes are shown in **Figure 1A**. The five genes with the highest mutation frequency in the overall samples were *PIK3CA* (40%), *KMT2D* (26%), *KMT2C* (26%), *LRP1B* (14%), and *FBXW7* (13%) genes. The gene mutations were mostly present in genes involved in the PI3K-AKT signaling pathway. Activation of *PIK3CA* mutations in cancer predominately leads to the dysregulation of the PI3K/AKT/mTOR pathway (**Figure 1B)**.

Differences in gene mutations between AC and SCC in our cohort

The distributions of gene mutations detected in AC and SCC are shown in **Figure 1C**. We observed a higher frequency of *PIK3CA* gene mutation in SCC compared with AC (49% vs. 15%, $p = 0.004$). Both *ERBB2* and *XPC* gene mutations were enriched in AC (0% vs. 12%, $p = 0.020$; 0% vs. 12%, $p = 0.020$, respectively). We also detected a trend toward more patients with SCC harboring *TERT* gene mutations (15% vs. 0%, $p = 0.057$), although the result did not reach statistical significance. *KRAS* gene mutation was more frequently observed in AC than in SCC (19% vs. 6%, $p = 0.110$).

Gene mutation differences between our cohort and TCGA cohort

The top 20 most frequently mutated genes in TCGA cohort are shown in **Figure 2A**. We observed a relatively consistent pattern of the frequently mutated genes in TCGA compared with our cohort. Notably, a higher *TERT* gene mutation frequency was detected in our cohort compared with TCGA cohort (12% vs. 1%, $p < 0.001$), especially in SCC (15% vs. 2%, $p < 0.001$) (**Figure 2B–C**).

We also compared gene mutations among the different histological subtypes between our cohort and TCGA cohort. In cervical SCC, the frequencies of *PIK3CA* gene mutation, *KMT2D* gene mutation and *FGFR3* gene mutation were significantly higher in our cohort compared with TCGA cohort (all $p < 0.05$) (**Figure 2C**). In cervical AC, significantly higher frequencies of gene mutations of *BRIP1* and *WRN*, which are targets of PARP inhibitor, were detected in our cohort compared with TCGA cohort (15% vs. 0%, $p = 0.017$; 12% vs. 0%, $p = 0.049$, respectively). In AC, we observed a lower *PIK3CA* mutation frequency in our cohort compared with TCGA cohort (15% vs. 37%, $p = 0.061$). The rates of *TP53* mutation and *ERBB2* mutation in our cohort were consistent with those in TCGA cohort (**Figure 2D**).

Differences in PD-L1 expression in cervical SCC and AC

Among the 98 CC samples, 75 patients were tested for PD-L1 expression, and the rate of positive PD-L1 expression was 72% (54/75) (table S1). Specimens were stratified into the following three groups (C1–3) based on the cut-off values for PD-L1 expression as defined in Methods: C1: CPS < 1; C2: 1≤ CPS < 10; and C3: CPS ≥ 10. The distribution of CC samples in each group was as follows: 28% (21/75) in C1; 35% (26/75) in C2; and 37% (28/75) in C3. The expression of PD-L1 in cervical AC and cervical SCC in the different subgroups is shown in **Figure 3A**. The rate of positive PD-L1 expression in cervical SCC was significantly higher than that in cervical AC (87% vs. 39%, $p < 0.001$), and the expression intensity of PD-L1 in cervical SCC was significantly higher than that in cervical AC (median CPS: 4 vs. 0, $p = 0.004$) (**Figure 3B**).

Differences in TMB in cervical SCC and AC

We defined TMB-high (TMB-H) as the top 25% of the TMB value of samples in our cohort. Among the 98 cervical cancer samples, 40% (39/98) were TMB-H, and 60% (59/98) were TMB-low. In comparing the difference in TMB between cervical SCC and AC, we found that SCC showed a significantly higher TMB compared with AC (median TMB: 5.830 vs. 1.670, $p < 0.001$) (**Figure 3C**).

KMT2C and *LRP1B* gene mutations are associated with increased TMB in cervical cancer

We next explored the correlation between gene mutation and TMB in cervical cancer by analyzing the top 15 most frequently mutated genes. *KMT2C* gene mutation in cervical cancer was associated with higher TMB levels compared with cervical cancers with wild-type *KMT2C* (median 10 vs. 2.5, $p < 0.001$). Similar results were observed with *LRP1B, PIK3CA, KMT2D, FBXW7, FAT1, LRP2, ARID1A, KMT2A, PRKDC, SPEN*, and *EP300* gene mutations (all $p < 0.05$) (**Figure 3D**).

To validate the association of gene mutations with TMB value, logistic regression was performed to analyze the factors that influence TMB-H. We analyzed the impact of histological subtype by dividing subtypes into SCC and non-SCC. The results indicated that the independent risk factors for TMB-H were KMT2C gene mutation and LRP1B gene mutation (all $p < 0.05$) (Table 1).

Correlation of PTEN mutations and PD-L1 expression with clinical outcomes

To analyze the correlation between genomic mutation and clinical outcomes, 29 patients were excluded, because gene mutations were not detected in 7 patients, 9 patients lost to follow-up and 13 patients received immunotherapy treatment. In 69 patients, PTEN mutations (7/69, 10.1%) were associated with poorer PFS (12.16 vs. 21.75 months, log-rank test $p = 0.0024$) and trend to worse OS (log-rank test $p = 0.096$) when compared to those without PTEN mutations (Figure 4A-B). For constructing the Cox proportional-hazard model, previously reported prognostic factors, such as age (continuous value), histological type (non-SCC vs. SCC), and stage, were used as adjustment factors during the analysis. In multivariate analysis, the PTEN mutations correlated with shorter PFS (hazard ratio 3.946, 95% CI, 1.500-10.380, $p = 0.050$) (Table 2). It suggests that PTEN mutations is an independent predictive factor of poorer clinical outcome in our patients.

A survival analysis was also performed in 75 patients by PD-L1 expression status. There was no significant difference between PD-L1-positive and -negative patients in OS (log-rank test $p = 0.1684$). However, shorter PFS were observed in patients with positive PD-L1 expression (log-rank test $p = 0.0267$) (**Figure S1**).

Discussion

There are limited data on the genomic alteration profile of cervical cancer in Chinese patients. In this study, cervical cancer samples from 98 Chinese patients were analyzed using NGS and immunohistochemistry to identify the characteristic features of gene alterations as well as TMB and PD-L1 expression. Significant differences in the personal genomic landscapes were detected across cervical cancer subtypes, and our results indicated that mutations of *KMT2C* and *LRP1B* genes were associated with higher independence of TMB. In addition, our findings suggest that PTEN mutations and PD-L1 expression are related to the prognosis of patients.

PD-L1 expression determined by immunohistochemistry is a validated biomarker with strong correlation to the response to immune checkpoint inhibitors. A previous report showed that the proportion of positive expression of PD-L1 (≥ 50%) in Chinese patients with non-small cell lung cancer might be different from those in Western countries patients [12]. In KEYNOTE 158, the overall response rate of cervical cancer patients with PD-L1-positive tumors (CPS≥1) to immunotherapy was 14.6% [4]. Previous studies have indicated that the efficacy of immune checkpoint inhibitor therapies is limited in cervical cancer patients [13]. According to previous reports, PD-L1 was expressed in 34.4%-96% of cervical cancer tissues expressed, while it was rarely found in normal cervical tissues [14]. Helen and others researchers reported the PD-L1 positivity in 83 of 154 (54%) of SCC and 7 of 49 (14%) of AC ($p < 0.001$) [15]. Consistent with

these data, we also noticed that PD-L1 expression was more frequent in SCC (87%) than in AC (39%), and the median CPS value in SCC (4) was higher than in AC (0). Same as previous studies, the result of our study showed that patients with PD-L1-negative have a better prognosis [16]. Nonetheless, the results of our prospective clinical study suggested that patients with high PD-L1 expression had better ORR (70.4% vs 33.3%; P = 0.041) and longer PFS (P = 0.014) than those with low expression in antivascular therapy combined with anti-PD-1 therapy (unpublished observations)[1].

Importantly, our study showed that the spectrum of genomic alterations in Chinese patients with cervical was roughly same as those in Western countries patients. In our study, *PIK3CA, KMT2C, KMT2D, LRP1B*, and *FBXW7* genes were the five genes with the highest mutation frequency in cervical cancer samples, which is mostly consistent with the results in TCGA. However, we found a substantially higher TERT alteration frequency in our study compared with TCGA. One reason for this result may be that the TERT mutations occurred in the promoter, and while WES is unable to detect the mutation in the promoter, we were able to detect the mutation using NGS. As far as we know, this is the first study to report a TERT promoter mutation in a Chinese population with cervical cancer. By WGS data, TERT hotspot mutations were found in 74% of primary glioblastoma cases in the previous study, and these mutations resulted in increased TERT RNA expression [17, 18]. The same hotspot mutation was also found in our research by NGS. These results indicate that TERT mutations may represent a therapeutic target for cervical cancer.

We also compared the frequency of mutations among histological subgroups of cervical cancer in this study. The AC prognosis is worse, which is probably because the differences in genetic mutations between subgroups. Our results revealed different characteristics of gene mutations in different pathological types of cervical cancer. *PIK3CA* gene alterations were considerably higher in SCC (49%) than in AC (15%). *PIK3CA* mutations occurred most frequently in the overall patient group, and the mutation rate in our study was higher than rates in previous studies in cervical carcinoma from the Netherlands (20%), France (27%), Latin America (28–33%), the USA (31%) and Norway (15%) [3, 19-22]. These differences may be related to differences in race, ethnicity, geography, tumor characteristics, or staging. *PIK3CA* is not only one of the PI3K/AKT/mTOR pathway members, but also is the most frequently mutated oncogene in human cancers[23]. *In vitro* studies have shown that the activated PI3K/Akt/COX-2 pathway may induce human AC HeLa cells to develop resistance to radiation [24]. Nusrat and other researchers recently reported that microsatellite stable colorectal cancer patients with *PIK3CA* mutations had benefited from immunotherapy [25]. Another study showed that PIK3CA mutations were connected with response of immunotherapy in cervical cancer [7]. However, only 32 patients were included in this analysis. Therefore, the relationship between *PIK3CA* mutations and immunotherapy needs to be further investigated in a study with a larger sample size. Our results also showed that the frequency of TERT gene mutation was significantly more frequent in SCC than AC.

We also reported for the first time the difference between the frequency of *FGFR3* mutations in cervical SCC and the TCGA database. Patients with *FGFR3* mutation may have relatively worse prognosis after chemotherapy and immune checkpoint inhibition [26]. The recent clinical trial of the selective FGFR1-3 inhibitor infigratinib for the treatment of FGFR3 altered metastatic breast cancer with second-line therapy

showed that the overall response rate was as high as 25% [27]. FGFR3 inhibitors may thus represent a potential therapeutic method for cervical cancer patients with *FGFR3* mutations. The mutation frequency of *KMT2D* gene was significantly higher in our group than that of TCGA. Wang et al. suggested that *KMT2D* deficiency make tumous more sensive to immune checkpoint blockade by enhancing tumor immunogenicity [28]. Our data indicated that KMT2D mutation was also related to a higher TMB in cervical cancer. Moreover, the results of a prospective study in our center suggested that patients with KMT2D mutations had better ORR ($p<0.05$) by anti-vascular therapy combined with anti-PD-1 therapy[2]. These results could help identify a sizeable patient subpopulation that may show sensitivity to immune checkpoint inhibition.

We also found significantly higher *BRIP1* and *WRN* gene mutations in AC compared with results in TCGA. *BRIP1* and *WRN* genes are important genes for homologous recombination repair and targets for PARP inhibitors. Thus, cervical cancers with *BRIP1* and *WRN* alterations may be impressible to PARP inhibitors.

The mutation frequencies of *KRAS, TP53* and *ERBB2* genes in AC in our group were consistent with TCGA. *ERBB2* mutation was enriched in AC in our cohort, which is consistent with other studies[22]. Retrospective studies identified *ERBB2* gene mutations in 35–6% of cervical cancers and reported that *ERBB2* gene mutations may be associated with a poor prognosis[22, 29, 30]. Although these results need to be further analyzed in larger sample sizes, our findings indicate that SCC and AC exhibit different molecular characteristics, which suggests that identifying specific targeted treatment strategies, such as PI3K and MEK inhibitors, may improve clinical outcomes for these patients.

We also examined TMB in the samples and performed statistical analysis to recognize gene mutations that were related to increased TMB. Previous studies showed that a high TMB value associated with improved therapeutic effect of immunotherapy in pan-cancer species, including cervical cancer [31]. The TMB value could be extrapolated to the burden measured by WES, and therefore it may be related to the predicted benefit of immune checkpoint inhibitors [32]. However, the use of WES to quantify TMB is currently not feasible in clinical practice. Rizvi et al. showed that the predicted value of TMB by NGS has a good correlation with that of WES [33]. A study verified the accuracy and the predictive value of TMB evaluated by the targeted gene panel we used in the current research [34]. In our study, we calculated TMB using an NGS-based gene panel test. We found that the degree of TMB was associated with the cervical cancer subtype: SCC had a significantly higher TMB than AC. Our findings indicate that the overall mutational landscapes have significant differences between cervical cancer subtypes. Thus, the differences of the responses to the cervical cancer subtypes in Chinese patients to immune checkpoint inhibitor could be caused by the variance in gene alterations, PD-L1 expression and TMB status across different subtypes. Further studies using a large sample size are required to solve this hypothesis.

Interestingly, we also found that genetic mutations in *KMT2C* and *LRP1B* are independent predictors of TMB-H. To our knowledge, this is the first finding that genetic mutations in *KMT2C* and *LRP1B* are independent predictors of TMB-H in cervical cancer. *KMT2C,* a member of the KMT2 family, has been provided as a tumor suppressor that is frequently altered in multiple cancers [35]. Non-small cell lung

cancer patients with mutated *KMT2C* gene have a higher TMB as well as PD-L1+/TMB-H, which are associated with a significantly longer median progression-free survival, compared with tumors with wild-type *KMT2C* [36]. In our cohort, the *KMT2C* gene was the third most frequent gene mutation in cervical cancer and also related to a higher TMB. These findings suggest the possibility of *KMT2C* gene mutation as a predictor of prognosis and response to immunotherapy and targeted therapy in cervical cancer. *LRP1B*, an endocytic low-density lipoprotein family receptor, is considered to be a candidate tumor suppressor, which binds to extracellular ligands. Alterations in *LRP1B* were associated with high TMB in melanoma and non-small cell lung cancer[37]. Although several studies have investigated the relation between *LRP1B* and TMB, no studies have provided definitive findings for cervical cancer. Here we found that mutations of *LRP1B* were associated with a higher TMB. *LRP1B*, as a result, may be defined as a predictive marker for the efficiency of CC immunotherapy.

The PTEN tumor suppressor is the second most commonly inactivated gene across cancer types. In a large cohort of Non-Small Cell Lung Cancer (NSCLC), PTEN loss was associated with poorer prognosis [38]. Loss of PTEN has been associated with resistance to BRAF inhibitor and decreased overall survival in melanoma [39, 40]. Similarly, the results of this study show that PTEN mutations are associated with worse clinical outcomes. However, the secondary analysis results of CLAP trial suggest that the PTEN alterations were associated with improved outcomes in patients with cervical cancer treated with PD-1 inhibitor combination therapy, it conferred longer PFS (p = 0.05) and trend toward increased OS (p = 0.08) [7]. PTEN mutations are associated with worse prognosis, but patients with PTEN mutations may benefit from anti-PD-1 combination therapy.

The study had several limitations. One limitation of this paper is the insufficient sample size. Another limitation is lack of direct data for the relation between gene mutations and clinical prognosis. Therefore, further research is required to investigate the connection between genetic alterations and efficiency of immunotherapy in Chinese patients with cervical cancer.

[1]Qin Xu, et al. Genomic profiling and PD-L1 expression of advanced cervical cancer to predict response to programmed death-1 inhibitor combination therapy: a secondary analysis of the CIBI308ALTER-C201 trial [abstract]. Gynecologic Oncology (unpublished observations).
[2] Qin Xu, et al. Genomic profiling and PD-L1 expression of advanced cervical cancer to predict response to programmed death-1 inhibitor combination therapy: a secondary analysis of the CIBI308ALTER-C201 trial [abstract]. Gynecologic Oncology (unpublished observations).

Conclusions

In conclusion, our study disclosed the gene landscape of cervical cancer in a relatively large sample numbers of Chinese patients. We also identified the different genomic landscapes and immune characteristics between cervical SCC and AC. These findings may provide a basis for potential therapeutic targets and improvement of treatment of cervical cancer patients. We also showed that cervical cancers with mutations in *KMT2C* and *LRP1B* genes have an increased TMB, and our data

indicate *KMT2C* and *LRP1B* gene mutations may serve as biomarkers to forecast therapeutic reaction. More important, one critical gene, PTEN, were identified to function as the prognostic marker for cervical patients' survival, providing insights for application of diagnostic and prognostic tools. We still need large sample prospective studies to verify our conclusions.

Abbreviations

SCC: squamous cell carcinoma; AC: adenocarcinoma; TCGA: the Cancer Genome Atlas; NGS: next-generation sequencing; TMB: tumor mutational burden; CPS: combined positive score; SNVs: Single nucleotide variants.

Declarations

Ethics approval and consent to participate

This project and protocols were approved by the Institutional Review Board of Fujian Provincial Cancer Hospital (Fuzhou, China), and all subjects signed informed consent forms (SQ2020-096-01). All patients gave informed consent for molecular analysis in blood and tissue, in accordance with the Declaration of Helsinki. All patients included in the study have consented for publication of the data generated by the analysis performed.

Consent for publication

Not applicable.

Availability of data and materials

TCGA database (Supplementary TCGA_PM2 data) and our cohort datasets (Supplementary mutation data) during this study are included in its supplementary data.

Competing interests

The authors declare that they have no competing interests, and all authors confirm its accuracy.

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Authors' contributions

Qin Xu and Jing Liu contributed to conception and design of the study. Zirong Li, Ting Lu and Junping Pan contributed to data collection. Zirong Li performed data statistical analysis. All authors contributed

to the article and approved the submitted version.

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References

1. Chen W, Zheng R, Baade PD, Zhang S, Zeng H, Bray F, Jemal A, Yu XQ, He J: Cancer statistics in China, 2015. CA: a cancer journal for clinicians 2016, 66(2):115-132.

2. Siegel RL, Miller KD, Jemal A: Cancer statistics, 2020. CA: a cancer journal for clinicians 2020, 70(1).

3. Integrated genomic and molecular characterization of cervical cancer. Nature 2017, 543(7645):378-384.

4. Chung HC, Ros W, Delord J-P, Perets R, Italiano A, Shapira-Frommer R et al: Efficacy and Safety of Pembrolizumab in Previously Treated Advanced Cervical Cancer: Results From the Phase II KEYNOTE-158 Study. Journal of clinical oncology : official journal of the American Society of Clinical Oncology 2019, 37(17):1470-1478.

5. Marabelle A, Fakih M, Lopez J, Shah M, Shapira-Frommer R, Nakagawa K et al: Association of tumour mutational burden with outcomes in patients with advanced solid tumours treated with pembrolizumab: prospective biomarker analysis of the multicohort, open-label, phase 2 KEYNOTE-158 study. Lancet Oncol 2020, 21(10):1353-1365.

6. Wang M, Fan W, Ye M, Tian C, Zhao L, Wang J et al: Molecular profiles and tumor mutational burden analysis in Chinese patients with gynecologic cancers. Scientific reports 2018, 8(1):8990.

7. Huang X, He M, Peng H, Tong C, Liu Z, Zhang X et al: Genomic profiling of advanced cervical cancer to predict response to programmed death-1 inhibitor combination therapy: a secondary analysis of the CLAP trial. Journal for immunotherapy of cancer 2021, 9(5).

8. Galic V, Herzog TJ, Lewin SN, Neugut AI, Burke WM, Lu Y-S, Hershman DL, Wright JD: Prognostic significance of adenocarcinoma histology in women with cervical cancer. Gynecologic oncology 2012, 125(2):287-291.

9. Eifel PJ, Burke TW, Morris M, Smith TL: Adenocarcinoma as an independent risk factor for disease recurrence in patients with stage IB cervical carcinoma. Gynecologic oncology 1995, 59(1):38-44.

10. Lee Y-Y, Choi CH, Kim T-J, Lee J-W, Kim B-G, Lee J-H, Bae D-S: A comparison of pure adenocarcinoma and squamous cell carcinoma of the cervix after radical hysterectomy in stage IB-IIA. Gynecologic oncology 2011, 120(3):439-443.

11. Kojima T, Shah MA, Muro K, Francois E, Adenis A, Hsu C-H et al: Randomized Phase III KEYNOTE-181 Study of Pembrolizumab Versus Chemotherapy in Advanced Esophageal Cancer. Journal of clinical oncology : official journal of the American Society of Clinical Oncology 2020, 38(35):4138-4148.

12. Aggarwal C, Abreu DR, Felip E, Carcereny E, Baas P: Prevalence of PD-L1 expression in patients with non-small cell lung cancer screened for enrollment in KEYNOTE-001, -010, and -024. Annals of Oncology 2016, 27(suppl_6).

13. Frenel J-S, Le Tourneau C, O'Neil B, Ott PA, Piha-Paul SA, Gomez-Roca C et al: Safety and Efficacy of Pembrolizumab in Advanced, Programmed Death Ligand 1-Positive Cervical Cancer: Results From the Phase Ib KEYNOTE-028 Trial. Journal of clinical oncology : official journal of the American Society of Clinical Oncology 2017, 35(36):4035-4041.

14. Enwere EK, Kornaga EN, Dean M, Koulis TA, Phan T, Kalantarian M et al: Expression of PD-L1 and presence of CD8-positive T cells in pre-treatment specimens of locally advanced cervical cancer. Modern pathology : an official journal of the United States and Canadian Academy of Pathology, Inc 2017, 30(4):577-586.

15. Heeren AM, Punt S, Bleeker MC, Gaarenstroom KN, van der Velden J, Kenter GG, de Gruijl TD, Jordanova ES: Prognostic effect of different PD-L1 expression patterns in squamous cell carcinoma and adenocarcinoma of the cervix. Modern pathology : an official journal of the United States and Canadian Academy of Pathology, Inc 2016, 29(7):753-763.

16. Gu X, Dong M, Liu Z, Mi Y, Yang J, Zhang Z et al: Elevated PD-L1 expression predicts poor survival outcomes in patients with cervical cancer. Cancer cell international 2019, 19:146.

17. Wang L-B, Karpova A, Gritsenko MA, Kyle JE, Cao S, Li Y et al: Proteogenomic and metabolomic characterization of human glioblastoma. Cancer cell 2021, 39(4).

18. Killela PJ, Reitman ZJ, Jiao Y, Bettegowda C, Agrawal N, Diaz LA et al: TERT promoter mutations occur frequently in gliomas and a subset of tumors derived from cells with low rates of self-renewal. Proc Natl Acad Sci USA 2013, 110(15):6021-6026.

19. Spaans VM, Trietsch MD, Peters AAW, Osse M, Ter Haar N, Fleuren GJ, Jordanova ES: Precise Classification of Cervical Carcinomas Combined with Somatic Mutation Profiling Contributes to Predicting Disease Outcome. PloS one 2015, 10(7):e0133670.

20. Wright AA, Howitt BE, Myers AP, Dahlberg SE, Palescandolo E, Van Hummelen P et al: Oncogenic mutations in cervical cancer: genomic differences between adenocarcinomas and squamous cell carcinomas of the cervix. Cancer 2013, 119(21):3776-3783.

21. Lou H, Villagran G, Boland JF, Im KM, Polo S, Zhou W et al: Genome Analysis of Latin American Cervical Cancer: Frequent Activation of the PIK3CA Pathway. Clinical cancer research : an official journal

of the American Association for Cancer Research 2015, 21(23):5360-5370.

22. Ojesina AI, Lichtenstein L, Freeman SS, Pedamallu CS, Imaz-Rosshandler I, Pugh TJ et al: Landscape of genomic alterations in cervical carcinomas. Nature 2014, 506(7488):371-375.

23. German S, Aslam HM, Saleem S, Raees A, Anum T, Alvi AA, Haseeb A: Carcinogenesis of PIK3CA. Hereditary cancer in clinical practice 2013, 11(1):5.

24. Xia S, Zhao Y, Yu S, Zhang M: Activated PI3K/Akt/COX-2 pathway induces resistance to radiation in human cervical cancer HeLa cells. Cancer Biother Radiopharm 2010, 25(3):317-323.

25. Nusrat M, Roszik J, Katkhuda R, Menter D, Kopetz S: Association of PIK3CA mutations (mut) with immune engagement and clinical benefit from immunotherapy in microsatellite stable (MSS) colorectal cancer (CRC) patients (pts). Journal of Clinical Oncology 2019, 37(15_suppl):3604-3604.

26. Robertson AG, Kim J, Al-Ahmadie H, Bellmunt J, Guo G, Cherniack AD et al: Comprehensive Molecular Characterization of Muscle-Invasive Bladder Cancer. Cell 2018, 174(4):1033.

27. Pal SK, Rosenberg JE, Hoffman-Censits JH, Berger R, Quinn DI, Galsky MD et al: Efficacy of BGJ398, a Fibroblast Growth Factor Receptor 1-3 Inhibitor, in Patients with Previously Treated Advanced Urothelial Carcinoma with Alterations. Cancer discovery 2018, 8(7):812-821.

28. Wang G, Chow RD, Zhu L, Bai Z, Ye L, Zhang F et al: CRISPR-GEMM Pooled Mutagenic Screening Identifies KMT2D as a Major Modulator of Immune Checkpoint Blockade. Cancer discovery 2020, 10(12):1912-1933.

29. Oaknin A, Friedman CF, Roman LD, D'Souza A, Brana I, Bidard F-C et al: Neratinib in patients with HER2-mutant, metastatic cervical cancer: Findings from the phase 2 SUMMIT basket trial. Gynecologic oncology 2020, 159(1):150-156.

30. Xiang L, Jiang W, Ye S, He T, Pei X, Li J et al: ERBB2 mutation: A promising target in non-squamous cervical cancer. Gynecologic oncology 2018, 148(2):311-316.

31. Yarchoan M, Hopkins A, Jaffee EM: Tumor Mutational Burden and Response Rate to PD-1 Inhibition. The New England journal of medicine 2017, 377(25):2500-2501.

32. Campesato LF, Barroso-Sousa R, Jimenez L, Correa BR, Sabbaga J, Hoff PM, Reis LFL, Galante PAF, Camargo AA: Comprehensive cancer-gene panels can be used to estimate mutational load and predict clinical benefit to PD-1 blockade in clinical practice. Oncotarget 2015, 6(33):34221-34227.

33. Rizvi H, Sanchez-Vega F, La K, Chatila W, Jonsson P, Halpenny D et al: Molecular Determinants of Response to Anti-Programmed Cell Death (PD)-1 and Anti-Programmed Death-Ligand 1 (PD-L1) Blockade in Patients With Non-Small-Cell Lung Cancer Profiled With Targeted Next-Generation Sequencing. Journal of clinical oncology : official journal of the American Society of Clinical Oncology 2018, 36(7):633-641.

34. Fang W, Ma Y, Yin JC, Hong S, Zhou H, Wang A et al: Comprehensive Genomic Profiling Identifies Novel Genetic Predictors of Response to Anti-PD-(L)1 Therapies in Non-Small Cell Lung Cancer. Clinical cancer research : an official journal of the American Association for Cancer Research 2019, 25(16):5015-5026.

35. Yang B, Li J, Li F, Zhou H, Shi W, Shi H et al: Comprehensive analysis of age-related somatic mutation profiles in Chinese young lung adenocarcinoma patients. Cancer medicine 2019, 8(4):1350-1358.

36. 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 immunotherapy in non-small cell lung cancer. Cancer medicine 2021, 10(7):2216-2231.

37. Chen H, Chong W, Wu Q, Yao Y, Mao M, Wang X: Association of Mutation With Tumor Mutation Burden and Outcomes in Melanoma and Non-small Cell Lung Cancer Patients Treated With Immune Check-Point Blockades. Frontiers in immunology 2019, 10:1113.

38. Kerr KM, Dafni U, Schulze K, Thunnissen E, Bubendorf L, Hager H et al: Prevalence and clinical association of gene mutations through multiplex mutation testing in patients with NSCLC: results from the ETOP Lungscape Project. Annals of oncology : official journal of the European Society for Medical Oncology 2018, 29(1):200-208.

39. Paraiso KHT, Xiang Y, Rebecca VW, Abel EV, Chen YA, Munko AC et al: PTEN loss confers BRAF inhibitor resistance to melanoma cells through the suppression of BIM expression. Cancer research 2011, 71(7):2750-2760.

40. Bucheit AD, Chen G, Siroy A, Tetzlaff M, Broaddus R, Milton D et al: Complete loss of PTEN protein expression correlates with shorter time to brain metastasis and survival in stage IIIB/C melanoma patients with BRAFV600 mutations. Clinical cancer research : an official journal of the American Association for Cancer Research 2014, 20(21):5527-5536.

Tables

Table 1. Univariate and multivariate logistic regression of factors influencing TMB-high in cervical cancer

Factor	Univariate		Multivariate	
	OR (95%CI)	*p*-value	OR (95%CI)	*p*-value
Histological subtypes (scc, non-scc)	5.358(1.837-15.623)	0.002	4.767(0.992-22.902)	0.051
KMT2C (mutant, wild)	11.368(3.744-34.520)	<0.001	13.160(2.814-61.553)	**0.001**
LRP1B (mutant, wild)	3.240(0.995-10.553)	0.051	5.981(1.311-27.291)	**0.021**
PIK3CA (mutant, wild)	2.657(1.149-6.148)	0.022	1.208(0.359-4.062)	0.760
FBXW7 (mutant, wild)	11.196(2.322-53.983)	0.003	5.338(0.819-34.764)	0.080
PRKDC (mutant, wild)	6.234(1.221-31.820)	0.028	1.966(0.240-16.122)	0.529
KMT2D (mutant, wild)	6.367(2.320-17.473)	<0.001	2.520(0.663-9.574)	0.175
EP300(mutant, wild)	6.234(1.221-31.820)	0.028	7.876(0.997-62.241)	0.050

Non-scc: non squamous cell carcinoma; scc: squamous cell carcinoma

Table 2. Univariate and multivariate analyses of clinical parameters on progression-free survival (PFS) (Cox regression) (N=69)

Variable	Univariate		Multivariate	
	HR (95% CI)	*p-value*	HR (95% CI)	*p-value*
Age	0.999(0.956-1.044)	0.955	0.979(0.934-1.026)	0.374
Histological types (non-SCC vs SCC)	2.031(0.771-5.353)	0.1433	1.702(0.621-4.660)	0.301
Stage (III-IV vs I-II)	1.864(0.905-3.842)	0.091	1.679(0.794-3.550)	0.175
PTEN (mutant, wild)	3.765(1.501-9.446)	**0.005**	3.946(1.500-10.380)	**0.005**

Figures

Figure 1

The landscape of frequently mutated genes in cervical cancer. (A) Oncoplot displaying the landscapes of frequently mutated genes. Genes are ordered according to the mutation frequency (left panel), and

different mutation types are indicated by the annotation bar (bottom). (B) Genomic mutations of PI3K/mTOR genes in cervical cancer. (C) Differences in mutations between adenocarcinoma (AC) and squamous cell carcinoma (SCC). *p < 0.05, **p < 0.01.

Figure 2

Landscapes of frequently mutated genes in cervical cancer from TCGA, and differences in mutations between our cohort and TCGA cohort. (A) Oncoplot displaying the landscapes of frequently mutated genes from TCGA cohort. Genes are ordered according to the mutation frequency (left panel), and different mutation types are indicated by the annotation bar (bottom). (B) Gene mutation differences between our cohort and TCGA cohort. Comparison of gene mutations between our cohort and TCGA cohort in cervical squamous cell carcinoma (SCC) (C) and in cervical adenocarcinoma (AC) (D).

Figure 3

PD-L1 expression and tumor mutation burden (TMB) in cervical cancer. (A) Distribution of PD-L1 expression in different histological subtypes of cervical cancer. Comparison of PD-L1 expression (B) and TMB (C) between cervical squamous cell carcinoma (SCC) and cervical adenocarcinoma (AC). C1: CPS <1; C2: 1≤ CPS <10; and C3: CPS ≥10. (D) Associations between TMB and mutations in *KMT2C* and *LRP1B, PIK3CA, KMT2D, FBXW7, FAT1, LRP2, ARID1A, EP300, KMT2A, PRKDC*, and *SPEN* genes. *p < 0.05; **p < 0.01; ***p < 0.001. WT, wild-type; MT, mutant type.

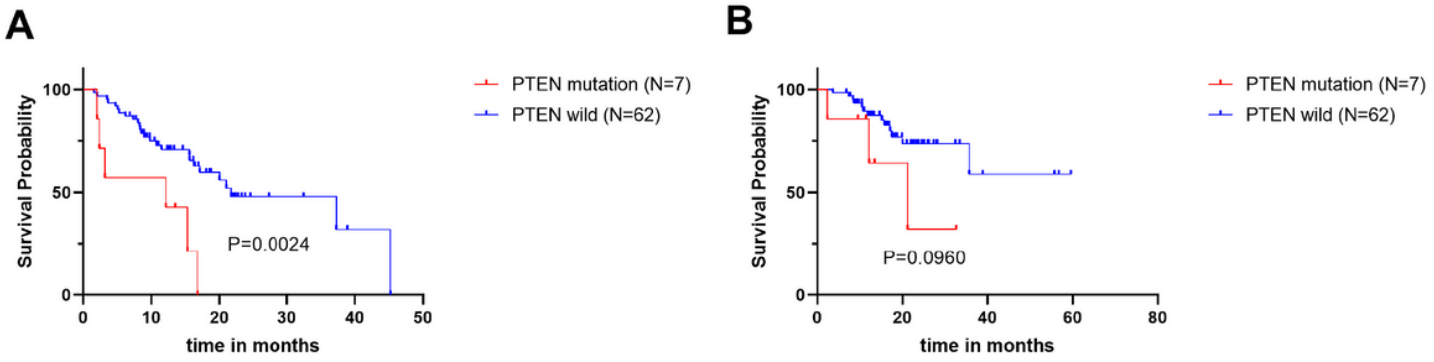

Figure 4

Kaplan-Meier estimated A] PFS and B] OS by PTEN mutational status.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryMaterial.docx
- SupplementaryTCGAPM2data.txt
- supplementarymutationdata.txt