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Exploring the Genomic Landscape and Therapeutic Targets of Metastatic/Recurrent Cervical Cancer Based on Deep Analysis of Large Panel Genes

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Abstract

Purpose: To delineate the genomic landscape and identify potential therapeutic vulnerabilities in metastatic or recurrent cervical cancer by integrating deep genomic sequencing data with comprehensive clinical and treatment response information.

Methods: We performed deep targeted Next-Generation Sequencing (NGS) on tumor specimens from 258 patients with metastatic or recurrent cervical carcinoma. Clinical demographics, treatment history, and outcomes were systematically curated from electronic medical records.

Results: The cohort had a median age of 54 years and included predominantly squamous cell carcinoma (SCC, 70.2%), adenocarcinoma (AC, 10.5%), and gastric-type adenocarcinoma (10.9%). The genomic landscape was characterized by high-frequency alterations in PIK3CA (41%), epigenetic regulators KMT2C (27%) and KMT2D (22%), and MUC16 (20%). A significant subset (27.5%) exhibited high tumor mutational burden (TMB-H, >10 mut/Mb). Distinct molecular profiles emerged by histology: SCCs were dominated by PIK3CA mutations (46.4%) and alterations in PI3K-AKT and chromatin remodeling pathways. In adenocarcinomas, TP53 and PIK3CA were most common. Notably, non-HPV-associated adenocarcinomas showed a trend toward higher mutation frequencies in genes like KRAS and had a significantly elevated TMB compared to HPV-associated tumors (mean 12.15 vs. 5.66 mut/Mb, $p<0.05$). Germline analysis identified 4% of patients carrying pathogenic variants in homologous recombination repair genes (e.g., PALB2, RAD51B). MUC16 and CREBBP mutations were significantly associated with radio resistance. Clinically, patients with TMB-H who received immunotherapy achieved a superior disease control rate (66.7%) compared to those with TMB-L (40.9%).

Conclusions: Significant molecular heterogeneity across histological subtypes, with distinct mutation patterns and pathway activations. Histology-specific therapeutic strategies and biomarker-driven approaches to optimize precision treatment in advanced cervical cancer.

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Introduction

Cervical cancer remains a leading cause of cancer-related mortality among women globally, with a disproportionate burden in regions lacking adequate screening and vaccination programs [1]. China faces particularly alarming epidemiological trends, characterized by high incidence and mortality rates (nearly 110,000 new cases and 60,000 deaths reported in 2022) [2]. early-stage disease is often curable, approximately 50% of patients present with locally advanced stages (IB3-IVA). For these patients, standard cisplatin-based Concurrent Chemoradiotherapy (CCRT) yields suboptimal outcomes, with a 5-year recurrence rate exceeding 50% and a Median Progression-Free Survival (PFS) often between 18 to 24 months [3,4]. The 5-year recurrence rate exceeds 50%, and this poor outcome is especially pronounced in patients with lymph node metastasis, who may experience PFS as short as 13.8 months [5]. The majority of patients with advanced disease will experience recurrence, and treatment options for recurrent/metastatic cervical cancer remain limited, primarily due to acquired resistance and a lack of biomarkers for precision therapy.

Genomic studies have begun to unravel the molecular complexity of cervical cancer. Early work identified recurrent mutations in genes such as PIK3CA, PTEN, and STK11 [6,7]. Subsequent whole-exome sequencing and The Cancer Genome Atlas (TCGA) project delineated subtype-specific alterations, confirming key drivers and identifying new significantly mutated genes while highlighting the role of APOBEC mutagenesis [8,9]. Recent studies in Chinese cohorts have further confirmed these patterns and explored associations with HPV integration and the immune landscape [10].

However, critical gaps persist. First, most genomic studies have focused on primary, early-stage, or unselected cohorts, leaving the genomic landscape of metastatic/recurrent disease—where treatment resistance is paramount—relatively unexplored. Second, there is frequently a disconnect between genomic data and detailed, longitudinal clinical information, hindering the translation of molecular findings into actionable biomarkers for predicting therapeutic response or resistance. Third, the functional and clinical implications of many alterations, especially in non-squamous subtypes, require validation in well-annotated clinical cohorts.

Therefore, the predictive value of genomic data in advanced cervical cancer is incompletely understood. This study aims to address these gaps by performing deep targeted sequencing on a real-world cohort of patients with metastatic/recurrent cervical cancer. We integrate multidimensional genomic data with comprehensive clinical and treatment response information to delineate subtype-specific molecular landscapes, identify potential therapeutic vulnerabilities, and validate candidate predictive biomarkers, with the ultimate goal of informing personalized treatment strategies.

Materials and methods

Sample and clinical data

All patients with advanced cervical cancer, recruited from Chongqing University Cancer Hospital. All aforementioned procedures were approved by the patients and their families, as well as the Ethics Committee of Chongqing University Cancer Hospital, complying with clinical standards and medical ethics requirements. High-throughput targeted sequencing was performed on 258 patients with metastatic and recurrent cervical cancer between 2017 and 2024, and their gene expression profiles were analyzed. However, due to sample availability/quality, germline testing was performed on 248 patients, and TMB analysis was available for 244 patients. Postoperative samples underwent standardized pre-processing, were embedded in paraffin blocks, and promptly subjected to subsequent testing.

Immunohistochemical analysis of PD-L1 expression

Immunohistochemistry (IHC) was used to detect PD-L1 expression levels in Formalin-Fixed and Paraffin-Embedded (FFPE) tissue samples. Detectable samples had to meet the criterion of containing over 100 viable tumor cells. PD-L1 expression was assessed using the Combined Positive Score (CPS), defined as the number of PD-L1 staining tumor cells (any membrane staining intensity), lymphocytes, and macrophages (membrane/cytoplasmic staining directly associated with tumor cells) divided by the total number of viable tumor cells, multiplied by 100. All necrotic cells, stromal cells, carcinoma in situ, and other immune cells (e.g., neutrophils, eosinophils, plasma cells) were excluded. Based on previous studies, CPS≥1 was defined as PD-L1 positive.

Tumor genomic sequencing

FFPE tissue samples (from primary or metastatic lesions) and matched blood samples (as controls) were collected from patients. Nucleic acids were extracted from both the tissue samples and white blood cells from the blood. Sequencing libraries were constructed, and a panel was used for targeted enrichment of cancer-related genes followed by sequencing. The high-throughput targeted sequencing kit and the standardized automated sample management and data analysis system provided by the BGI sequencing platform were used for raw data filtering and bioinformatic analysis.

The bioinformatic analysis pipeline consisted of two main stages: First, the quality of the sequencing data was assessed, including statistics on sequencing error rate, data volume, and alignment rate, to evaluate whether the library construction and sequencing processes met the required standards. Samples passing quality control proceeded to subsequent analysis; otherwise, they were re-processed or subjected to additional sequencing. The second stage involved variant calling and analysis. High-quality sequences were aligned to the reference genome to detect germline mutations and somatic mutations in the samples, followed by further interpretation of the identified variants.

Microsatellite instability

Microsatellite Instability (MSI) status was evaluated using the LuMSI software, which analyzes 1137 relevant characteristic loci based on NGS data. A random forest algorithm employing 100 decision tree classifiers was used. The prediction results from each decision tree were obtained, and a vote was conducted to classify the sample as MSI-H or MSI-L/MSS based on the number of characteristic loci supporting each category. LM_State: The LM_MSI status of the sample. A threshold of 50 was used; the category with over 50 votes determined the final status. Support_MSI-H: Number of decision trees supporting the MSI-H classification. Support_MSI-L/MSS: Number of decision trees supporting the MSI-L/MSS classification.

Tumor mutational burden calculation

Tumor Mutational Burden (TMB) was defined as the number of somatic mutations per megabase (Mb) of the targeted sequencing region, with the unit being Muts/Mb. As standardized methods for TMB calculation and classification are not yet uni-

versally established, samples with a TMB value exceeding the upper quartile (25%) in this study were defined as TMB-High (TMB-H). Although the clinically established TMB-H threshold is often defined as ≥10 muts/Mb, the upper quartile in our cohort corresponded to 6.81 muts/Mb; this value was therefore used to stratify patients for exploratory analysis. Somatic mutations included in the TMB calculation comprised Single Nucleotide Variants (SNVs) and short insertions or deletions (Indels), including synonymous mutations. Reported driver mutations (mutations closely related to cancer therapy, diagnosis, or prognosis, including hotspot mutations, drug target mutations, oncogene-activating mutations, and tumor suppressor gene-inactivating mutations) were excluded from the TMB calculation.

Human papillomavirus testing

Sanity HPV Genotyping (RT-PCR, Sanity, China, Changsha) is used for the qualitative in vitro detection of HPV in exfoliated cervical epithelial cells. It covers the following 23 genotypes: 6, 11, 16, 18, 26, 31, 33, 35, 39, 42, 43, 45, 51, 52, 53, 56, 58, 59, 66, 68, 73, 81, and 82. Using specific primers and fluorescent probes in combination with Polymerase Chain Reaction (PCR) and Taqman technology, the assay amplifies HPV DNA fragments in multiple reaction tubes to enhance fluorescence signals. The viral genotypes are ultimately determined based on the Ct value (the number of cycles required to reach the threshold).

GO and KEGG analysis

Gene Ontology (GO; http://www.geneontology.org/) and the Kyoto Encyclopedia of Genes and Genomes (KEGG; www.genome.jp/kegg/) databases were used as the bioinformatics resource and comprehensive database, respectively, for functional and pathway classification of the genes covered in the sequencing panel. The DAVID database (the Database for Annotation, Visualization and Integrated Discovery; https://david.ncifcrf.gov/) was referenced for analyzing the biological processes associated with the mutated genes and for obtaining KEGG pathway enrichment results. A P-value<0.05 was considered statistically significant.

Statistical analysis

All statistical analyses in this study were performed using SPSS software (version 25.0). The enrichment of genomic alterations across different histological subtypes was analyzed using Fisher's exact or χ^2 tests, as appropriate, with nominal P values reported. A P-value of less than 0.05 was considered statistically significant. Comparative analysis of gene mutation frequency was performed across the histological subgroups. For patients enrolled in clinical trials, the Objective Response Rate (ORR) was evaluated per RECIST v1.1. Progression-Free Survival (PFS) was defined as the time from treatment initiation to the first occurrence of disease progression (as determined by the investigator according to RECIST v1.1) or death from any cause, whichever occurred first. Disease Control Rate (DCR) was defined as the proportion of patients achieving a best overall response of Complete Response (CR), Partial Response (PR), or Stable Disease (SD), assessed per Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1.

Results

Cohort characteristics of cervical cancer

This study included a total of 258 patients, with a median age of 54 years (range: 22–81). Squamous cell carcinoma was the predominant histologic type (181 cases, 70.16%), followed by adenocarcinoma (27 cases, 10.47%) and gastric adenocarcinoma (28 cases, 10.85%). There were 22 cases of special histological types of cervical cancer, encompassing entities such as small cell neuroendocrine carcinoma, mucin-producing adenocarcinoma, clear cell carcinoma, et cetera. Sequencing samples were predominantly collected from primary tumor sites (73.64%), with metastatic biopsies and blood-derived samples each comprising 13.18% of the total. 82.6% of the tumors were positive for high-risk HPV. Upon initial diagnosis, 79.46% of cases were classified as locally advanced disease, and distant metastasis was present in 20.54% of the cohort. PD-L1 was positive (CPS≥1) in 88.1% of 42cases (5 CPS<1, 28 CPS 1-10, 16 CPS≥10). Squamous cell carcinoma has the highest positivity rate, 95.7% (22/23), HPV-related adenocarcinoma was 85.7% (6/7). Among the entire cohort of 258 patients, the most common treatment modalities were chemotherapy (n=236, 91.47%) and radiotherapy (n=230, 89.15%). A subset of patients underwent surgical intervention, with staging surgery performed in 64 (24.81%) cases and radical surgery in 63(24.42%). Patient demographic data are summarized in Table 1.

Mutational Landscape of metastatic/recurrent cervical cancer

Our study uncovered distinct mutational profiles across pathological cancer types, underscoring their unique molecular pathogenesis, including 921 somatic variations, 117 copy number variations and 49 fusion mutations of genes. Among the 258 patients, somatic alterations primarily amplifications were most frequently identified in PIK3CA (41%), KMT2C (27%), KMT2D (22%), MUC16 (20%), and FBXW7 (16%). These were followed by recurrent alterations in LRP1B (14%), EP300 (14%), and TP53 (14%). High-frequency mutations were predominantly missense variants (Figure 1A/B). Notably, KMT2D and KMT2C exhibited high mutation frequencies, primarily nonsense mutations predicted to result in truncated proteins. The co-occurrence rate with PIK3CA gene accounts for 42% of KTM family mutations. In addition, splicing mutations are extremely high in TERT, indicating that mutations at the starting codon of the gene are more active. And the co-occurrence of this gene with PIK3CA and KTM family gene mutations is as high as 80.8%. Copy number variations are mainly amplified, such as PIK3CA amplification accounting for 18.8%, SOX2 amplification accounting for 15.38%, MCL1 amplification accounting for 12.82% (Figure 1C). There are 49 fusion gene mutations, including DCUN1D1-ASCC3, FGFR3-TACC3, FMO4-TOP1, (Figure 1D). The CUN1D1-ASCC3 and FGFR3-TACC3 fusion genes are prominent.

In Squamous Cell Carcinoma (SCC), characteristic driver gene mutations were prominent. Among these, PIK3CA was the most frequently mutated gene, observed in 46.4% of cases, suggesting that activation of the PI3K–AKT signaling pathway is a key mechanism in SCC pathogenesis. Additionally, frequent mutations in epigenetic regulators such as KMT2C (30.4%) and KMT2D (27.1%) indicated that dysregulation of chromatin remodeling plays a crucial role in SCC development. The high mutation rates of FBXW7 (21.0%) and FAT1 (16.0%) further delineated the distinct molecular subtype of SCC. In contrast, HPV-associated Adenocarcinomas (AC) and Gastric-type Adenocarcinoma (GAC) displayed more similar mutational landscapes, which were markedly different from that of SCC. Both gland-derived subtypes were dominated by TP53 mutations (AC: 37.0%; GAC: 35.7%), underscoring the central role of p53 tumor suppressor dysfunction in the pathogenesis of adenocarcinomas. More-

over, KRAS mutations were more prevalent in GAC (21.4%) than in AC (11.1%), implying that RAS–MAPK signaling activation may represent a specific characteristic of gastric-type adenocarcinoma, as shown in Figure 2. Several genes, including BRCA2, ARID1A, and NF1, exhibited low yet consistent mutation rates universally, indicating their role as shared oncogenic events rather than drivers of any specific pathological subtype. In contrast, comparing whole-exome sequencing data from 32 cases of HPV-associated adenocarcinomas and 33 cases of non-HPV-associated adenocarcinomas (including gastric-type and clear cell carcinomas), we found a substantial overlap at the genetic level between the two groups which were markedly different from that of SCC.TP53 (HPV^+: 31.3% vs. HPV^-: 45.5%) and PIK3CA (HPV^+: 15.6% vs. HPV^-: 24.2%) were the most frequently mutated genes, followed by other shared high-frequency mutations such as ARID1A, KMT2C, and FAT3. Although the non-HPV-associated group showed a trend toward higher mutation frequencies in most genes (e.g., KRAS: 6.3% vs. 21.2%), chi-square tests revealed that none of these differences reached statistical significance (all $p>0.05$). However, the non-HPV-associated group exhibited a significantly higher Tumor Mutational Burden (TMB) than the HPV-associated group (mean mutations per sample: 12.15 vs. 5.66). These results suggest that while HPV-positive and HPV-negative adenocarcinomas may share some core oncogenic pathways, their molecular pathogenic mechanisms differ: HPV-associated adenocarcinomas are characterized by more specific driver genetic alterations, whereas non-HPV-associated adenocarcinomas demonstrate greater genomic instability and potential susceptibility to immunotherapy.

Germline pathogenic/likely pathogenic variants

Among 258 patients with advanced cervical cancer, 10 out of the 248 who underwent germline genetic testing were identified as carrying suspected or known germline Pathogenic/Likely Pathogenic (P/LP) variants (4%). Two of these cases had a family history of cancer: one carried an MSH2 mutation associated with Lynch syndrome, with two first-degree relatives diagnosed with endometrial and colon cancers; the other carried a BARD1 mutation related to Homologous Recombination Deficiency (HRD), with two first-degree relatives affected by cervical and gastrointestinal cancers.

Among the 10 germline variants detected, five were splice-site mutations, three were frameshift mutations, one was a nonsense mutation, and one was a missense mutation. Genes including PALB2, RAD51B, BARD1, and FANCA—which are highly implicated in the Homologous Recombination Repair (HRR) pathway were involved.

Predictive biomarkers for chemoradiotherapy sensitivity

Patients were stratified based on their response to chemoradiotherapy into a group achieving Complete or Partial Response (CR+PR) and a group with Stable or Progressive Disease (SD+PD). A chi-square test was performed to compare mutation frequencies between these groups. We identified significant associations between specific gene mutations and treatment response. MUC16 and CREBBP mutations were significantly associated with favorable response to chemoradiotherapy. Among all genes analyzed, only MUC16 ($p=0.0004$) and CREBBP ($p=0.021$) exhibited mutation frequencies that reached the conventional threshold of statistical significance ($p<0.05$). The mutation rate of MUC16 was significantly higher in the CR+PR group (24.26%) compared to the SD+PD group (5.36%). Mutations in CREBBP were detected exclusively in the CR+PR group (8.28%) and were absent in the SD+PD group. Furthermore, four genes showed a trend toward significance, including PTPRD ($p=0.050$), KMT2A ($p=0.050$), PIK3R1 ($p=0.061$), and CASP8 ($p=0.082$). All exhibited higher mutation rates in the CR+PR group, although these differences did not reach statistical significance. The biological and clinical relevance of these trends warrants further validation in larger cohorts.

Of the 258 patients included in this study, TMB data were available for 244 (94.6%), among whom 177 were classified as TMB-L and 67 as TMB-H. The prevalence of TMB-H across histological subtypes was as follows: 33.1% (57/172) in squamous cell carcinoma, 17.4% (4/23) in adenocarcinoma, 14.8% (4/27) in gastric-type adenocarcinoma, 0% (0/8) in small cell neuroendocrine carcinoma, 0% (0/5) in mucin-producing adenocarcinoma, 20.0% (1/5) in clear cell carcinoma, and 33.3% (1/3) in other subtypes. Immunotherapy was administered to 34 patients, 12 (17.9%) of the TMB-H patients, resulting in a Disease Control Rate (DCR) of 66.7% (8/12). The responses consisted of one Complete Response (CR, 8.3%), four Partial Responses (PR, 33.3%), and three cases of Stable Disease (SD, 25.0%). In the TMB-L group, 22 (12.4%) patients received immunotherapy, achieving a DCR of 40.9% (9/22), which included two Complete Responses (CR 9.1%), three Partial Responses (PR 13.6%), and four Stable Disease cases (SD 18.2%).

The signaling pathway of cervical cancer

GO and KEGG analyses were performed on the mutated genes across different pathological subtypes to investigate their signaling pathways, as shown in Figure 3. Top five KEGG enriched pathways for high-frequency genes in cervical cancer are: Human papillomavirus infection, Breast cancer, Human T-cell leukemia virus 1 infection, MicroRNAs in cancer, and Hepatocellular carcinoma (Figure 3A). Based on the GO enrichment analysis results (Figure 3B), the enrichment profile of this group of cervical squamous cell carcinoma samples is relatively specific. Compared to adenocarcinoma and gastric-type adenocarcinoma, it more closely resembles a typical “HPV-driven” model. Its molecular characteristics primarily revolve around DNA damage stress and transcriptional reprogramming: The significant enrichment of the cellular response to UV pathway (reflecting DNA damage stress) and the activation of the phosphatidylinositol 3-kinase/protein kinase B signal transduction pathway (involving cell survival signaling) together constitute an adaptive mechanism for coping with genomic instability. Simultaneously, the coordinated enrichment of pathways such as positive regulation of transcription by RNA polymerase II, chromatin remodeling, and transcription coactivator activity indicates that the gene expression regulatory system drives extensive transcriptional reprogramming through DNA-binding proteins (DNA binding) within the nucleus (nucleus, nucleoplasm). Viral infection causes DNA damage, disrupts replication and transcription, leading to genomic instability. KEGG pathway analysis further extends this finding (Figure 3C): The enrichment of Cancer: specific types (gene count: 11) and Infectious disease: Viral (gene count: 7) pathways directly corroborates the HPV infection-driven, cervical cancer-specific carcinogenic mechanism. The activation of Signal transduction (gene count: 9) and Cell growth and death (gene count: 6) pathways provides supplementary functional evidence for PI3K-Akt signaling transduction and cell cycle regulation. The enrichment of the Drug resistance: antineoplastic (gene count: 4) pathway suggests a potential risk for the development of clinical drug resistance, while Cellular community - eukaryotes (gene count: 6) reveals

abnormalities in cell junctions and communication within the tumor microenvironment. Together, the two analyses delineate a multidimensional pathological network in cervical squamous cell carcinoma involving viral carcinogenesis, DNA damage, host transcriptional reprogramming -uncontrolled proliferation, cell cycle dysregulation, drug resistance development.

The molecular characteristics of cervical adenocarcinoma are concentrated in the deep-level chromatin remodeling processes associated with viral infection. Cells exhibit widespread abnormalities in transcriptional regulation, including significant enrichment of terms such as "transcription initiation-coupled chromatin remodeling," "chromatin remodeling," and "RNA polymerase II-specific DNA binding transcription factor binding," which drive transcriptional reprogramming. Concurrently, p53 tumor suppressor function is specifically inactivated through "MDM2/MDM4 family protein binding" and "p53 binding", rather than activating its downstream DNA damage apoptotic response. Furthermore, the activation of the "phosphatidylinositol 3-kinase/Protein Kinase B signal Transduction" (PI3K-Akt pathway) provides continuous survival signals for cells, while the lack of enrichment for "negative regulation of cell growth" confirms uncontrolled proliferation. KEGG pathway analysis further reveals, from a macro perspective, its upstream etiology and core clinical challenges: namely, the enrichment of the "Infectious disease: viral" pathway (gene count: 6) provides direct evidence for the HPV carcinogenic mechanism, the consequences of which are reflected in pathways like "Cancer: overview" (gene count: 11). Most importantly, the high enrichment of the "Drug resistance: antineoplastic" pathway (gene count: 10) elucidates the molecular basis for its potential chemotherapy resistance. The enrichment of the "Signal transduction" (gene count: 8) and "Cell growth and death" (gene count: 6) pathways corroborates the GO analysis results, jointly constructing a disease model driven by the virus, involving synergistic action of multiple pathways, and ultimately leading to malignant proliferation and drug resistance.

Analysis of the molecular functional profile of cervical Gstric-type Adenocarcinoma (GAS) indicates that its enrichments are more scattered and complex, presenting a core characteristic of endogenous inactivation of the p53 pathway. It is not only more sensitive to DNA positive/negative transcriptional regulation patterns but also more prone to affecting transcription factor activity and activating driver gene mutations: The most highly enriched terms, MDM2/MDM4 family protein binding and intrinsic apoptotic signaling pathway in response to DNA damage by p53 class mediator, reveal that p53 function is completely disrupted through the cell's own regulatory mechanisms rather than viral attack. Meanwhile, the strong enrichment of vasculature development predicts active tumor angiogenesis, while abnormalities in transcription initiation and chromatin remodeling indicate widespread dysregulation of epigenetic mechanisms cooperatively driving carcinogenesis. KEGG pathway analysis further extends and confirms this discovery: The enrichment of "Cancer: overview" and "Specific types" pathways (gene counts 9 and 11) summarizes the aforementioned carcinogenic phenotypes. Interestingly, the high enrichment of the "Infectious disease: Viral" pathway (gene count: 10) in GAS is speculated not to imply HPV infection, but rather to reveal that GAS, through endogenous mechanisms such as TP53 mutation/MDM2 overexpression, mimics the carcinogenic effect of HPV viral degradation of p53 at the molecular functional level, achieving "phenotypic convergence." Ultimately, these mechanisms collectively lead to the activation of the "Drug resistance: antineoplastic" pathway (gene count: 6), providing the molecular basis for its inherent chemotherapy resistance.

Clinical actionability

We annotated and stratified the therapeutic actionability of genomic alterations in 258 patients according to the OncoKB knowledge base. Our analysis revealed that a substantial proportion of the cohort (34%, 89/258) harbored at least one potentially actionable genetic alteration classified as OncoKB Level 3B, suggesting alternative therapeutic avenues in specific clinical contexts.

The distribution of higher-level actionable alterations was further delineated. Notably, all patients with OncoKB Level 1 alterations—established biomarkers for standard-care targeted therapies—concomitantly exhibited either high Tumor Mutational Burden (TMB-H) or Microsatellite Instability-High (MSI-H) phenotypes (with 64 and 2 patients identified as TMB-H and MSI-H in the cohort, respectively). Furthermore, we identified 8 patients with OncoKB Level 2 alterations and 11 with OncoKB Level 3 alterations. The latter group included 8 cases of ERBB2 amplification and 3 cases of NRG1 alterations, predominantly involving the HER family signaling pathway.

The translational impact of genomic profiling was evidenced by clinical trial enrollment. Among the 33 patients enrolled in therapeutic clinical trials, 4 were successfully matched to trials based on genomic testing results. All four of these matched patients were TMB-H and subsequently received PD-1/L1 immune checkpoint inhibitor therapy, underscoring the clinical utility of this biomarker. Of particular significance, we documented an exceptional responder case. A patient with HER2 protein expression of 1+ (IHC) and lacking canonical HER2 (ERBB2) mutations or gene amplification achieved durable remission exceeding 5 years following treatment with a HER2-targeting Antibody-Drug Conjugate (ADC). This remarkable outcome suggests the existence of previously uncharacterized sensitivity mechanisms to ADC agents. Further investigation into these mechanisms may help expand the population of patients who could benefit from such therapies in the future.

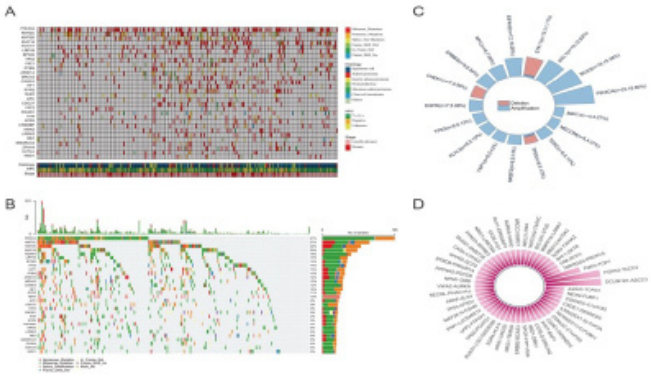

Figure 1: (A) Heatmap of Specific Gene Mutations in 258 Cervical Cancer Cases, Annotated with Key Features. **(B)** Genomic Waterfall Plot of 258 Cervical Cancer Cases. **(C)** Copy Number Variation (CNV) Landscape in 258 Cervical Cancer Cases. **(D)** Summary of Fusion Gene Alterations in 258 Cervical Cancer Cases.

Table 1: Clinical characteristics of cervical cancer patients.

Age	
• Median	54
• Range	22~81
Histology (n, %)	
• Squamous cell	181(70.16%)
• Adenocarcinoma	27(10.47%)
• Gastric adenocarcinoma	28(10.85%)
• Other	22(8.54%)
Sequencing sample type	
• Primary	190(73.64%)
• Metastatic	34(13.18%)
• Blood	34(13.18%)
High-risk HPV status (n, %)	
• Positive	143(55.43%)
• Negative	30(11.63%)
• Unknown	85(32.95%)
FIGO stage at initial diagnosis	
• Locally advance	205
• Distant	43
Disease status	
• NED	33
• AWD	86
• DOD	73
• Loss to follow up	66
PDL-1 CPS	
• ≤1	5
• 1-10	21
• ≥10	16
• Unknow	216
The initial treatment (n, %)	
• Radical surgery	63(24.42%)
• Staging surgery	64(24.81%)
• Radiotherapy	230(89.15%)
• Chemotherapy	236(91.47%)
The treatment received after recurrence (n, %)	
• Radiotherapy	28(31.82%)
• Chemotherapy	59(67.05%)
• Immunotherapy	34(38.6%)
• surgery	7(7.95%)

Abbreviations: AWD: Alive with disease; NED: No evidence of disease; DOD: Dead of disease; HPV: Human papillomavirus.

Discussion

This study delineates the genomic landscape of metastatic or recurrent cervical cancer through deep targeted sequencing of 258 patients, revealing pronounced molecular heterogeneity across histological subtypes and identifying distinct therapeutic vulnerabilities. By integrating comprehensive genomic data with detailed clinical annotations, our work reinforces the paradigm that advanced cervical cancer comprises a spectrum of molecularly distinct diseases dictated by histology and underlying driver alterations [11]. This granular understanding moves beyond a one-size-fits-all approach and provides a tangible roadmap for precision oncology.

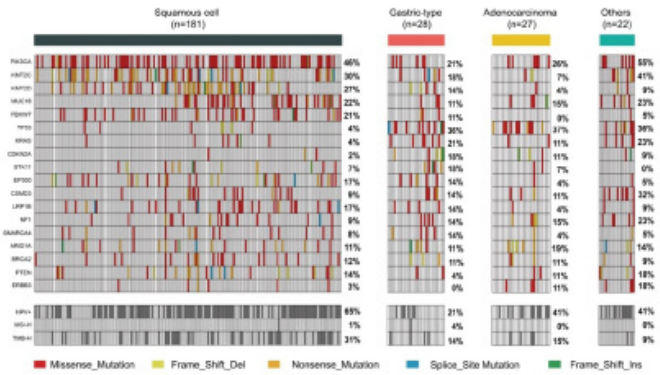

Figure 2: Heatmap of Cervical Squamous Cell Carcinoma, Adenocarcinoma, Gastric-type Adenocarcinoma, and Other Subtypes.

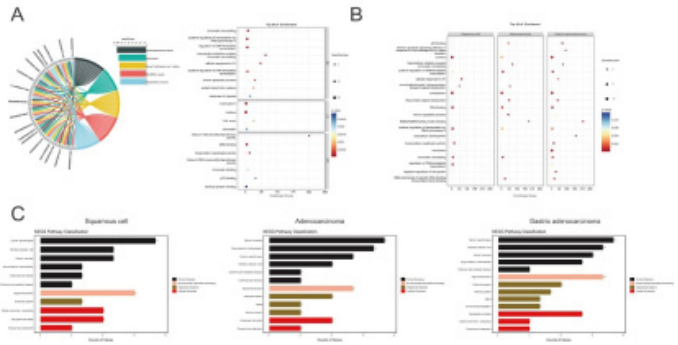

Figure 3: (A) Chord Diagram and Hierarchical Bubble Plot from GO Analysis of High-Frequency Genes in 258 Cervical Cancer Cases. **(B)** Enrichment Grouping Analysis of the Top 20 Genes in Squamous Cell Carcinoma, Adenocarcinoma, and Gastric-type Adenocarcinoma. **(C)** Bar Plots of KEGG Pathways for High-Frequency Genes in Cervical Cancer, Squamous Cell Carcinoma, and Adenocarcinoma.

Subtype-specific molecular pathogenesis and therapeutic implications

Our analysis unveiled fundamentally divergent mutational spectra between the major histological subtypes. Squamous Cell Carcinomas (SCCs), constituting the majority of our cohort, were predominantly driven by activating mutations in PIK3CA (46.4%). This high frequency underscores the PI3K-AKT-mTOR signaling pathway as a central oncogenic mechanism in SCC pathogenesis, a finding consistent with prior genomic studies, including The Cancer Genome Atlas (TCGA) project, which identified PIK3CA as a core significantly mutated gene in cervical cancer [7]. Importantly, these mutations often involve specific hotspots known to confer constitutive pathway activation. Coupled with frequent alterations in epigenetic regulators KMT2C/D and tumor suppressors like FBXW7 and FAT1, this profile paints a picture of SCC as a disease of concurrent signaling pathway activation and dysregulated chromatin remodeling [11]. The clinical corollary is clear: this molecular subset represents a prime candidate for targeted therapeutic strategies. PI3K/AKT pathway inhibitors, such as the PI3Kα-specific agent alpelisib, have demonstrated preliminary clinical activity in PIK3CA-mutant gynecological cancers, providing a strong rationale for their focused evaluation in advanced cervical SCC [12].

In stark contrast, adenocarcinomas exhibited a different driver landscape. Both usual-type (AC) and the aggressive Gastric-type Adenocarcinomas (GAC) were characterized by high frequencies of TP53 mutations (AC: 37.0%; GAC: 35.7%), highlighting the central role of p53 pathway dysfunction across

glandular subtypes [13,14]. Notably, we observed a higher KRAS mutation rate in GAC (21.4%) compared to conventional AC (11.1%). This subtype-specific enrichment suggests that aberrant RAS-MAPK signaling may be a critical and distinguishing pathogenic feature of GAC [10]. From a therapeutic perspective, this identifies RAS-MAPK signaling as a potential vulnerability in GAC. Preclinical models and emerging clinical data from other KRAS-mutant cancers support the exploration of MEK inhibitors, potentially in rational combination with other agents, to overcome adaptive resistance and achieve meaningful efficacy in this poor-prognosis subtype [15].

The roles of epigenetic dysregulation and HPV status

Beyond canonical signaling pathways, our data highlight the profound importance of epigenetic dysregulation. Genes encoding chromatin modifiers, particularly KMT2C (27%) and KMT2D (22%), were among the most frequently altered in the entire cohort, with peak frequencies observed in SCC. This aligns with their recognition as key tumor suppressors in cervical cancer and across various squamous cell carcinomas [16,17]. The high prevalence of predicted loss-of-function mutations in these genes suggests that disrupted histone methylation and aberrant chromatin remodeling are fundamental to carcinogenesis, offering a compelling rationale for exploring epigenetic therapies.

Further stratification of adenocarcinomas by HPV status yielded critical insights with direct implications for immunotherapy. While the core somatic mutation profiles of HPV-positive and HPV-negative adenocarcinomas showed substantial overlap at the gene level, a stark difference emerged in genomic instability. HPV-negative tumors exhibited a significantly higher tumor mutational burden (mean TMB: 12.15 vs. 5.66 mut/Mb in HPV-positive tumors). This elevated TMB, a recognized source of neoantigens, provides a strong molecular rationale for considering immune checkpoint inhibitors in this subgroup [10,18]. However, this opportunity is nuanced. Evidence suggests that HPV-negative cervical cancers, particularly adenocarcinomas, often harbor a more profoundly immunosuppressive tumor microenvironment—a "cold" phenotype [19,20]. Therefore, our findings argue for a biomarker-driven strategy wherein high TMB identifies patients with a theoretical basis for response, but combination regimens—perhaps pairing immune checkpoint blockade with agents that modulate the tumor microenvironment—are rationally designed to convert immunologically "cold" tumors.

Germline susceptibility and emerging predictive biomarkers

Our study extends the molecular characterization beyond the tumor genome to the germline. We identified that 4% of patients carried pathogenic or likely pathogenic germline variants, predominantly in genes associated with the Homologous Recombination Repair (HRR) pathway, such as PALB2 and RAD51B [21,22]. This finding uncovers a previously underappreciated hereditary component in a subset of cervical cancer patients. It underscores the importance of considering genetic counseling and germline testing, not only for familial risk assessment but also for therapeutic decision-making. The presence of an HRR deficiency establishes a theoretical foundation for the use of PARP inhibitors based on the principle of synthetic lethality, a concept supported by early preclinical studies in cervical cancer models [23-25].

In the quest for predictive biomarkers, our analysis linked specific somatic alterations to treatment outcomes. Mutations in MUC16 and CREBBP were significantly associated with a favorable response to chemoradiotherapy. The biological plausibility of these associations is noteworthy: MUC16 mutations may alter the immune-permissive landscape of the tumor [26,27], while CREBBP mutations could impair DNA damage repair and chromatin-mediated survival signals following radiation [10]. These findings offer novel mechanistic clues to radiosensitivity and resistance. On the immunotherapy front, our real-world data confirmed the utility of TMB as a pragmatic biomarker: TMB-high patients who received immune checkpoint inhibitors achieved a superior disease control rate (66.7%) compared to TMB-low patients (40.9%), validating TMB's role in patient selection within this specific disease context [28].

Pathway insights, clinical actionability, and future directions

Functional enrichment analysis provided a systems-level view of subtype-specific pathophysiology. SCCs exhibited signatures of an HPV-driven model, with coordinated activation of DNA damage response, PI3K-Akt signaling, and transcriptional reprogramming pathways [29,30]. Adenocarcinomas showed distinct enrichment for chromatin remodeling complexes and specific patterns of p53 pathway inactivation [31]. Gastric-type adenocarcinoma presented a unique profile suggestive of endogenous p53 pathway ablation, achieving a "phenotypic convergence" with the effects of HPV E6 oncoprotein [32,33]. These pathway insights deepen our understanding of divergent carcinogenic processes.

From a translational standpoint, our annotation using the OncoKB knowledge base confirmed the clinical actionability of findings: 34% of patients harbored genomic alterations with potential treatment implications [34]. These included alterations in genes like ERBB2 and NRG1, pointing to targeted therapy opportunities. The successful matching of several TMB-high patients to immunotherapy clinical trials, alongside an exceptional responder to a HER2-targeted antibody-drug conjugate, serves as proof-of-concept for the practical utility of comprehensive molecular profiling in guiding personalized therapy [28,34].

Conclusion

This study presents a comprehensive genomic characterization of metastatic and recurrent cervical cancer through deep analysis of large panel sequencing data from 258 patients. Our findings underscore the significant molecular heterogeneity across histological subtypes and delineate distinct genomic landscapes that correlate with clinical phenotypes and therapeutic vulnerabilities.

Key insights include:

Subtype-Specific Driver Mutations: Squamous Cell Carcinomas (SCCs) are predominantly driven by mutations in PIK3CA (46.4%) and epigenetic regulators (KMT2C/D), highlighting the central role of PI3K-AKT signaling and chromatin remodeling. In contrast, adenocarcinomas, particularly gastric-type, are characterized by frequent TP53 and KRAS mutations, suggesting divergent oncogenic pathways.

HPV Status and Genomic Instability: HPV-negative adenocarcinomas exhibit higher Tumor Mutational Burden (TMB) compared to HPV-positive tumors, indicating greater genomic instability and potential responsiveness to immunotherapy, al-

beit within an immunosuppressive microenvironment that may require combinatorial strategies.

Actionable Alterations and Biomarkers: A substantial proportion (34%) of patients harbored clinically actionable genomic alterations, with ERBB2 amplifications and NRG1 fusions representing promising targets. Biomarkers such as TMB-H and specific gene mutations (MUC16, CREBBP) were associated with treatment response, supporting their utility in guiding therapy.

Germline Susceptibility: Pathogenic germline variants in homologous recombination repair genes (e.g., PALB2, RAD51B) were identified in 4% of patients, underscoring the importance of genetic counseling and potential for PARP inhibitor therapy in selected cases.

Pathway Dysregulation: Functional enrichment analyses revealed subtype-specific activation of oncogenic pathways, including PI3K-AKT, RAS-MAPK, and chromatin remodeling networks, which may inform the development of targeted and combination therapies.

In summary, our integrated genomic and clinical analysis reinforces that metastatic/recurrent cervical cancer comprises molecularly distinct diseases defined by histology and underlying genetic alterations. These findings provide a rationale for biomarker-driven treatment strategies and highlight the need for prospective validation of candidate biomarkers in clinical trials. Future efforts should focus on longitudinal genomic profiling, functional validation of novel alterations, and the development of tailored therapeutic approaches that integrate targeted agents with immunotherapy to improve outcomes in this challenging patient population.

Limitations and future directions

This study has several limitations. The relatively small sample size of rare subtypes (e.g., small cell neuroendocrine carcinoma) limits the statistical power for subtype-specific analyses. clinical follow-up data were incomplete for some patients, precluding detailed survival analysis based on genomic alterations. The lack of paired primary and recurrent tumor samples prevents direct analysis of genomic evolution during disease progression. Future studies should validate these findings in larger, multicenter cohorts with longitudinal sampling to explore dynamic genomic changes and their impact on treatment response. Additionally, functional studies are needed to confirm the role of novel biomarkers (e.g., MUC16, CREBBP mutations) in radiotherapy response and to develop combination therapies targeting subtype-specific pathways.

Author declarations

Disclosure

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