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Brief Communication

Unveiling pathogenic mutations in BRCA1 and BRCA2 genes across head and neck squamous cell carcinoma patients via next generation sequencing

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Abstract: Head and Neck Squamous Cell Carcinoma (HNSC) presents a formidable challenge in the field of oncology due to its aggressive nature and the limited therapeutic options available. In this study, our primary focus was on the Pakistani HNSC patient population, aiming to investigate germline oncogenic mutations within the BRCA1 and BRCA2 genes via Next Generation Sequencing (NGS) and explore their clinical implications. We sought to understand the functional consequences of these mutations via RT-qPCR and Immunohistochemistry (IHC) techniques. The key discovery of our research lies in the identification of three pathogenic mutations, including two within BRCA1 (p.Cys274Ter and p.Glu272Ter) and one within BRCA2 (p.Met1Val), among Pakistani HNSC patients. These mutations previously associated with an increased risk of various cancers. What sets our study apart is the uniqueness of these pathogenic mutations, absent in HNSC patients from other populations. This suggests a distinct genetic profile in Pakistani HNSC patients, possibly contributing to their susceptibility to this malignancy. Furthermore, our research revealed elevated expression levels of BRCA1 and BRCA2 genes in HNSC samples harboring pathogenic mutations, offering insights into mechanisms driving tumor progression in HNSC. Importantly, we identified significant enrichment of BRCA1/2 genes in pathways related to cancer development within the KEGG database. Finally, in our quest to explore therapeutic avenues, we systematically analyzed drugs targeting up-regulated and mutated BRCA1/2 genes, identifying promising candidates for tailored treatment modalities in HNSC. In conclusion, our study reveals the unique genetic profile of HNSC in Pakistani patients, featuring unique pathogenic mutations in BRCA1 and BRCA2 genes. These mutations offer promise as valuable diagnostic markers and potential therapeutic targets.

Keywords: HNSC, next generation sequencing, germline mutations

Introduction

Head and Neck Squamous Cell Carcinoma (HNSC) represents a diverse and complex group of malignancies that originate in the mucosal surfaces of the upper aerodigestive tract, including the oral cavity, pharynx, and larynx [1, 2]. HNSC is a global health concern, accounting for a significant portion of cancer-related morbidity and mortality worldwide [3, 4]. HNSC is influenced by several key risk factors. The primary contributors include tobacco use (smoking or chewing), excessive alcohol consumption, and human papillomavirus (HPV) infection, particularly high-risk HPV strains [5-7]. Additionally, exposure to environmental carcinogens, such as asbestos and certain chemicals, poor oral hygiene, and a diet lacking in fruits and vegetables, can elevate the risk of HNSC [8-10]. Despite advances in diagnosis and treatment, the etiology and genetic basis of HNSC remain multifaceted and incompletely understood.

In recent years, there has been a growing recognition of the importance of germline oncogenic

Table 1. An overview of HNSC patient's characteristics in the present study

Sr. no	Characteristics	Sample count (n)
1	Sex	
	Male	0
	Female	22
2	Age	
	>60	0
	<60	22
3	Treatment	
	Pre-treatment	22
	Post-treatment	0

mutations in contributing to the risk and pathogenesis of various cancer types, including HNSC [11-13]. These germline mutations, often associated with well-known cancer predisposition genes, can significantly influence an individual's susceptibility to cancer development [13-15]. Among these genes, Breast Cancer Susceptibility Gene 1 (BRCA1) and Breast Cancer Susceptibility Gene 2 (BRCA2) have emerged as key players not only in breast and ovarian cancers but also in an expanding spectrum of malignancies, including those of the head and neck [16, 17].

The BRCA1 and BRCA2 genes, originally identified for their role in hereditary breast and ovarian cancer syndromes, have provided pivotal insights into the genetic underpinnings of cancer susceptibility [18]. Germline mutations in these genes are strongly associated with an elevated risk of developing breast, ovarian, and prostate cancers [19, 20]. However, recent studies have illuminated their involvement in a broader range of malignancies, prompting the exploration of their significance in HNSC [21, 22].

Next-Generation Sequencing (NGS), also known as high-throughput sequencing, has revolutionized the field of genetics and genomics, particularly in the context of germline mutation detection [23-26]. NGS is a powerful and versatile technology that enables the rapid and cost-effective sequencing of entire genomes, exomes, or targeted gene panels [24]. NGS allows for the simultaneous analysis of multiple genes, facilitating the identification of pathogenic mutations associated with inherited diseases, including cancer predisposition syndromes [27-30].

This manuscript embarks on a comprehensive investigation into the presence and implications of germline oncogenic mutations in BRCA1 and BRCA2 within the context of HNSC. Our research endeavors to bridge the knowledge gap surrounding the role of these genes in HNSC susceptibility and pathogenesis.

Methodology

Sample collection

HNSC tissue samples were collected to identify germline BRCA1/2 mutations in a cohort of 22 HNSC patients. These patients were enrolled and underwent resection at the Mufti Mehmood Memorial Teaching Hospital, D.I.G Khan, Pakistan between March 2020 and June 2022 and had received a confirmed diagnosis of HNSC. Prior to sample collection, ethical approval was obtained from the PARC, Pakistan. A summary of the clinicopathological characteristics of the patients is provided in **Table 1**. Prior to tissue sampling, written informed consent was obtained from each participant.

Inclusion and exclusion criteria

The inclusion and exclusion criteria for the selection of patients in this study were carefully defined to ensure the relevance and integrity of the research. Inclusion criteria encompassed patients who had received a histologically confirmed diagnosis of HNSC, were 18 years of age or older, provided written informed consent, and were prospectively enrolled at the hospital. Exclusion criteria were established to exclude patients with a history of unrelated malignancies, incomplete clinical data, those who did not provide informed consent, individuals below the age of 18, and patients with contraindications or conditions that could compromise the accuracy of NGS.

Nucleic acid extraction

DNA isolation from the HNSC tissues was done using organic method [31], while RNA was extracted via TRIzol method [32] and quantified using Qubit assay according to manufacturer's instructions (Thermo Fisher Scientific, USA).

Next generation sequencing (NGS)

The genomic DNA isolated was appropriately diluted to achieve the required concentration for polymerase chain reaction (PCR)-based library preparation. Targeted amplification of the coding regions and splicing sites of BRCA1 (NM 007294) and BRCA2 (NM 000059) genes was carried out using the AmpliSeq for Illumina BRCA Panel. The uniquely indexed libraries specific to the BRCA panel were then prepared following the guidelines outlined in the AmpliSeq for Illumina BRCA Panel Reference Guide. Subsequently, paired-end sequencing by synthesis was performed using the MiSeq sequencer from Illumina, located in San Diego, CA, USA.

The base quality and amplicon coverage of the raw sequencing reads were analyzed using the local run manager of the MiSeq sequencer. Following this, the cleaned reads, meeting the quality threshold of a phred score greater than 30, were aligned to the human reference genome hg19/GRCh37. This alignment was accomplished using the BWA-MEM Whole-Genome Aligner, version 0.7.9a-isis-1.0.2. Subsequently, mismatched calls were identified as mutations using the Pisces Variant Caller, version 5.2.9.23. These genetic mutations were then characterized and annotated with the assistance of the Illumina Annotation Engine, version 2.0.11-0-g7fb24a09.

Interpretation of the mutations

The interpretation of genetic mutations followed the guidelines established by the American College of Medical Genetics (ACMG) and the Association for Molecular Pathology (AMP) [33]. To classify these mutations into categories such as pathogenic, likely pathogenic, variants of uncertain significance (VUS), likely benign, or benign, a combination of in-silico prediction tools and curated external databases was employed. In line with the results generated by in-silico prediction software, including SIFT (http://sift.jcvi.org/), PolyPhen-2 (http://genetics.bwh.harvard.edu/pph2/), and Mutation Taster (http://www.mutationtaster.org/), the functional impact of these variants on the BRCA1 and BRCA2 protein products was determined. Furthermore, the ClinVar database (https://www.ncbi.nlm.nih.gov/clinvar/) was consulted to assess the clinical significance of the respective mutations.

Sanger sequencing

Sanger sequencing was carried out using SeqStudio Genetic Analyzer System (Thermo Fisher Scientific) and BigDye Terminator 3.1 Cycle Sequencing Kit (Thermo Fisher Scientific) to validate the identified pathogenic mutations in BRCA1/2 genes detected through NGS analysis.

Analysis of mutational frequencies

The Genome Aggregation Database (gnomAD) stands as a valuable and extensively employed genetic repository. It consolidates and disseminates exome and genome sequencing data across diverse populations, providing researchers with the means to investigate genetic variations and their frequencies [34]. In the context of this study, we harnessed the gnomAD database to assess the frequencies of the observed BRCA1/2 mutations within the Asian population.

Survival analysis (Kaplan-Meier)

We employed Kaplan-Meier survival curves [35] to evaluate the influence of mutations in BRCA1/2 genes on the overall survival (OS) of patients with HNSC. Graphical representations of the survival curves illustrate the probability of survival over time for two distinct cohorts: HNSC patients with pathogenic mutations identified in BRCA1/2 genes and those without such mutations. To ascertain the statistical significance of the observed differences in survival, we utilized the log-rank test, a widely accepted statistical method for comparing survival distributions. This test examined whether the disparities in OS between the two groups held statistical significance, thereby offering valuable insights into the prognostic implications of these mutations in the context of HNSC.

Reverse transcription quantitative polymerase chain reaction (RT-qPCR)

The RNA extracted was transformed into complementary DNA (cDNA) using High-Capacity cDNA Reverse Transcription Kits provided by Applied Biosystems. Following this, reverse transcription quantitative polymerase chain

reaction (RT-qPCR) was performed using Platinum PCR SuperMix High Fidelity kits from Life Technologies. Each reaction underwent three replicates to ensure precision. To quantify mRNA expression levels, the following formula was applied: mRNA expression level = 2^(-ΔΔCq) [36]. An independent samples t-test was utilized to identify differences in expression between the specified groups.

Receiver operating curve generation

Based on the RT-qPCR expression data, ROC curves of BRCA1/2 expression levels were generated with the help of SRPLOT web source (https://bioinformatics.com.cn/srplot).

Immunohistochemistry (IHC)

Tissue sections underwent deparaffinization, and antigen retrieval was achieved through heat treatment in an EDTA (ethylenediaminetetraacetic acid) solution at pH 8.0. Protein expression levels of the mutated genes in HNSC tissue samples were assessed using 4-μm-thick sections from formalin-fixed, paraffin-embedded (FFPE) specimens. Monoclonal antibodies against BRCA1 (EPR19433, abcam) and BRCA2 (EPR23442-43, abcam) were employed, and the Ventana BenchMark XT staining system (Roche, Tokyo, Japan) was utilized for the staining process. In this analysis, non-pathogenic mutated tissue samples served as the comparative reference. A pathologist determined the positivity of tumors based on the presence of nuclear staining in tumor tissue or negativity when nuclear staining was absent. Protein expression was assessed with consideration of staining intensity.

cBioPortal analysis

cBioPortal stands as an accessible and user-friendly platform specifically crafted for cancer genomics research [37]. It provides a comprehensive set of robust tools tailored for the exploration of intricate cancer genomic datasets. This platform empowers researchers to seamlessly visualize, analyze, and comprehend genetic alterations present in diverse cancer types, thereby advancing our insights into the disease. In this particular study, we harnessed the capabilities of this database to scrutinize clinically important mutations within the TCGA HNSC dataset.

Enrichment analysis

Metascape serves as a versatile bioinformatics resource with extensive applications in KEGG (Kyoto Encyclopedia of Genes and Genomes) and GO (Gene Ontology) analyses [38]. It simplifies the investigation of biological pathways, functions, and molecular interactions within extensive datasets. In the context of this study, we leveraged the capabilities of this valuable tool to conduct GO and KEGG analyses on the mutated BRCA1/2 genes. A significance level of $P<0.05$ was applied as the threshold criterion for the functional enrichment analysis.

Drug prediction analysis

DrugBank represents a comprehensive and highly respected resource within the realm of pharmacology [39]. It functions as a pivotal repository of data concerning drugs, drug targets, and drug interactions, encompassing both approved pharmaceuticals and investigational compounds. In the context of this research, we employed the DrugBank database to investigate drugs that regulate the expression of mutated BRCA1/2 genes.

Results

Next generation sequencing (NGS) and mutation detection

In this study, all 22 enrolled patients with HNSC were female, and their ages ranged from 21 to 55 years, with average age of 34 years. These patients were diagnosed with primary HNSC. Among the HNSC samples analyzed, 12 mutations were identified in the BRCA1 gene, while 8 mutations were found in the BRCA2 gene. Notably, all of these mutations received a high mutation quality score of 100. The sequencing reads exhibited an impressive coverage rate of 98.6%, and the average Quality score (Q30) reached 97%. Upon conducting in silico analysis and ClinVar-based interpretation, it was determined that there were 2 pathogenic mutations (17%) and 10 benign mutations (83%) in the BRCA1 gene (**Figure 1** and **Table 2**). Similarly, within the BRCA2 gene, the analysis revealed 1 pathogenic mutation (14%) and 7 benign mutations (86%) within the studied cohort (**Figure 1** and **Table 2**).

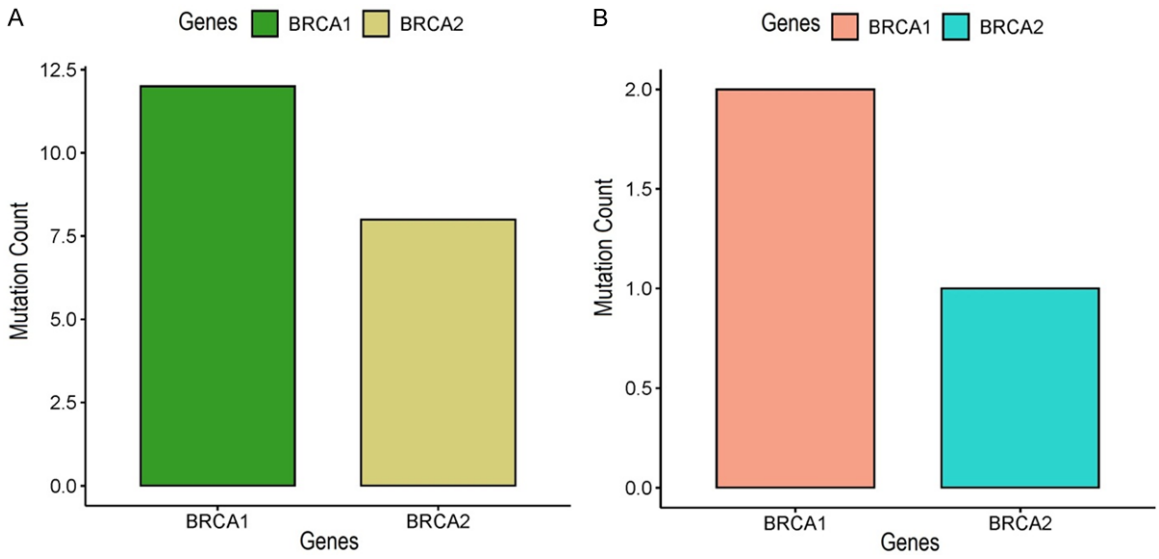

Figure 1. Total count of overall detected mutations and pathogenic mutations in BRCA1/2 across HNSC patients via WES. (A) An overall count of detected mutations in BRCA1/2 genes across HNSC patients, and (B) A count of detected pathogenic mutations in BRCA1/2 genes across HNSC patients.

Table 2. Count and type of mutations observed in BRCA1/2 genes across HNSC patients

Sr. no	Gene	NM:c.DNA	Protein	Nature (ClinVar)	Nature (In silico analysis)	No. patients
1	BRCA1	NM_007294.4:c.822T>A	p.Cys274Ter	Pathogenic	DC	5
2		NM_007294.4:c.814G>T	p.Glu272Ter	Pathogenic	DC	5
3		NM_007294.4:c.5198A>G	p.Asp1733Gly	Benign	Non-DC	11
4		NM_007294.4:c.5158A>G	p.Thr1720Ala	Benign	Non-DC	11
5		NM_007294.4:c.5117G>C	p.Gly1706Ala	Benign	Non-DC	4
6		NM_007294.4:c.5044G>A	p.Glu1682Lys	Benign	Non-DC	2
7		NM_007294.4:c.4985T>C	p.Phe1662Ser	Benign	Non-DC	4
8		NM_007294.4:c.4955T>C	p.Met1652Thr	Benign	Non-DC	9
9		NM_007294.4:c.4913A>T	p.Glu1638Val	Benign	Non-DC	12
10		NM_007294.4:c.4910C>T	p.Pro1637Leu	Benign	Non-DC	1
11		NM_007294.4:c.4883T>C	p.Met1628Thr	Benign	Non-DC	12
12		NM_007294.4:c.4840C>T	p.Pro1614Ser	Benign	Non-DC	1
13	BRCA2	NM_000059.4:c.1A>G	p.Met1Val	Pathogenic	DC	5
14		NM_000059.4:c.502C>A	p.Pro168Thr	Benign	Non-DC	9
15		NM_000059.4:c.865A>C	p.Asn289His	Benign	Non-DC	9
16		NM_000059.4:c.978C>A	p.Ser326Arg	Benign	Non-DC	11
17		NM_000059.4:c.1040A>G	p.Gln347Arg	Benign	Non-DC	6
18		NM_000059.4:c.1123C>T	p.Pro375Ser	Benign	Non-DC	2
19		NM_000059.4:c.1141G>A	p.Asp381Asn	Benign	Non-DC	2
20		NM_000059.4:c.1151C>T	p.Ser384Phe	Benign	Non-DC	8

DC = Disease causing.

Interestingly, all three pathogenic mutations, encompassing two mutations in BRCA1 (p.Cys274Ter and p.Glu272Ter) and one in the BRCA2 gene (p.Met1Val) were identified among the same five HNSC patients (as detailed in **Table 2**). Later on, these three pathogenic mutations were also confirmed among five HNSC patients using Sanger sequencing. Due

to the direct association of pathogenic mutations with disease development, the subsequent part of our study focused on analyzing the frequencies and functional consequences of these pathogenic mutations among HNSC patients.

Analyzing frequencies of the observed pathogenic mutations in Asian HNSC patients

We conducted an analysis of the frequencies of the three observed pathogenic mutations in BRCA1 (p.Cys274Ter and p.Glu272Ter) and BRCA2 (p.Met1Val) genes using the gnomAD database to assess their prevalence in Asian HNSC patients. Strikingly, our results revealed that these mutations had not been previously documented in HNSC patients, as they exhibited zero frequencies in the gnomAD database. Consequently, it is evident that these mutations represent a distinct genetic profile unique to Pakistani HNSC patients, suggesting their potential utility as population-specific biomarkers.

Analysis of BRCA1/2 mutations in the cancer genome atlas HNSC samples

Subsequently, we extended our investigation to analyze mutations in BRCA1/2 genes within the TCGA HNSC dataset using cBioPortal. Our analysis revealed a diverse spectrum of BRCA1/2 mutations in TCGA HNSC patients (**Figure 2**). Remarkably, the three pathogenic mutations identified in BRCA1 (p.Cys274Ter and p.Glu272Ter) and BRCA2 (p.Met1Val) genes in our study were conspicuously absent among the TCGA HNSC patients. This stark contrast reaffirms the rarity and distinctiveness of these mutations specific to Pakistani HNSC patients, further emphasizing their potential as unique genetic markers in this population.

Reverse transcription-quantitative polymerase chain reaction analysis to document expression profile of BRCA1/2 genes

We proceeded with an expression analysis of the BRCA1/2 genes via RT-qPCR, stratifying our sample cohort into two distinct groups. The first group consisted of HNSC samples (n = 17) lacking pathogenic mutations in BRCA1 (p.Cys274Ter and p.Glu272Ter) and BRCA2 (p.Met1Val) genes, while the second group included HNSC samples (n = 5) harboring pathogenic mutations. This analysis aimed to elucidate the functional implications of the observed pathogenic mutations. Our results distinctly revealed significantly elevated BRCA1/2 gene expression levels within the samples containing pathogenic mutations (**Figure 3A**), underscoring the potential functional consequences associated with these mutations.

Subsequently, we assessed the discriminative potential of BRCA1 and BRCA2 gene expressions. We observed a substantial area under the curve (AUC) of 0.776 (p-value <0.05) for BRCA1 expression (**Figure 3B**), mirroring a similar discernible pattern for BRCA2 expression with an AUC of 0.760 (p-values <0.05). These findings emphasize the notable sensitivity and specificity exhibited by these genes, highlighting their promising utility as discriminatory markers.

Immunohistochemical evaluation of BRCA1/2 protein expression

In this phase of our research, we conducted an immunohistochemical (IHC) analysis to evaluate the protein expression of BRCA1 and BRCA2. For this purpose, we utilized two distinct tissue samples: one obtained from an HNSC patient devoid of pathogenic mutations in BRCA1 (p.Cys274Ter and p.Glu272Ter) and BRCA2 (p.Met1Val) genes, and the other from an HNSC patient harboring pathogenic mutations in BRCA1/2 genes. The results of our analysis, as depicted in **Figure 4**, clearly demonstrated significantly higher staining intensities of BRCA1/2 in the HNSC sample with pathogenic mutations compared to the reference sample showing lower frequencies. These findings strongly suggest an up-regulation of BRCA1/2 genes associated with the presence of pathogenic mutations, providing further valuable insights into their functional consequences.

Enrichment analysis of BRCA1/2 genes

Subsequently, we conducted comprehensive GO and KEGG enrichment analyses to gain insights into the functional implications of BRCA1/2 genes. In the realm of Gene Ontology (GO), our analysis revealed significant enrichments of BRCA1/2 genes in various Cellular Component (CC) terms, such as “lateral element”, “synaptonemal structure”, “synaptone-

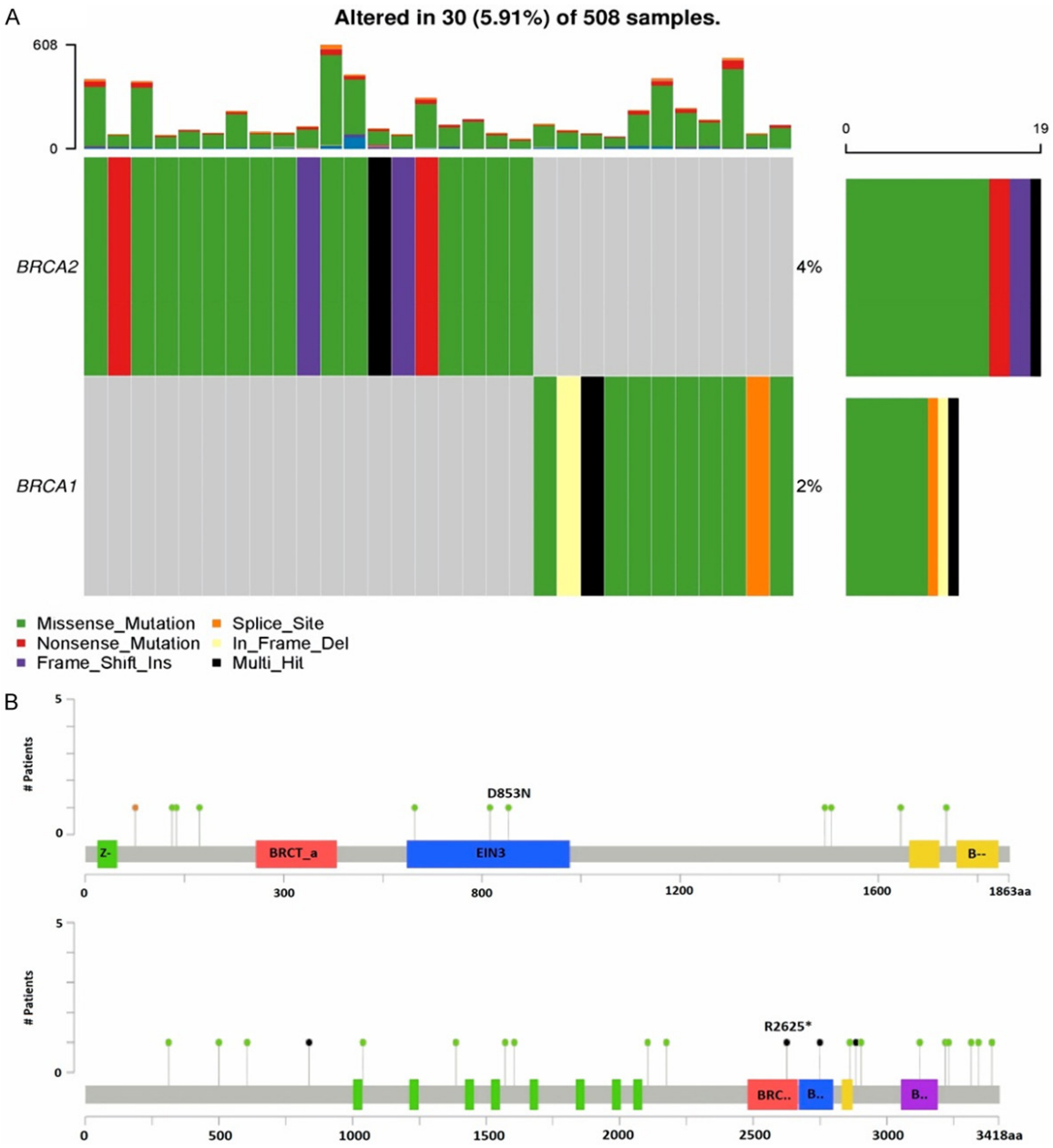

Figure 2. Oncoplot and lollipop plot-based visualization of the observed BRCA1/2 mutations across TCGA HNSC patients. Two rows showed percentage of HNSC samples which are positive for BRCA1/2 mutations, and lollipop plots highlighted amino acid change due to mutation at the protein level.

mal complex", "condensed nuclear chromosome", and "nuclear chromosome" etc. (**Figure 5A**). Within Molecular Function (MF) terms, enrichments were observed in categories like "tubulin binding", "H4 histone acetyltransferase activity", "gamma tubulin binding", and "acetyltransferase activity" etc. (**Figure 5B**). Furthermore, Biological Process (BP) terms exhibited enrichments related to "DNA damage response", "signal transduction by p53 class mediator resulting in transcription of P21 class mediator", "DNA damage response", "signal transduction resulting in transcription", and "histone H3-acetylation" etc. (**Figure 5C**). In the context of KEGG pathways, BRCA1/2 genes were notably enriched in pathways encompassing "homologous recombination", "fanconi anemia pathway", "breast cancer", and "platinum drug resistance in Cancer" etc. (**Figure 5D**).

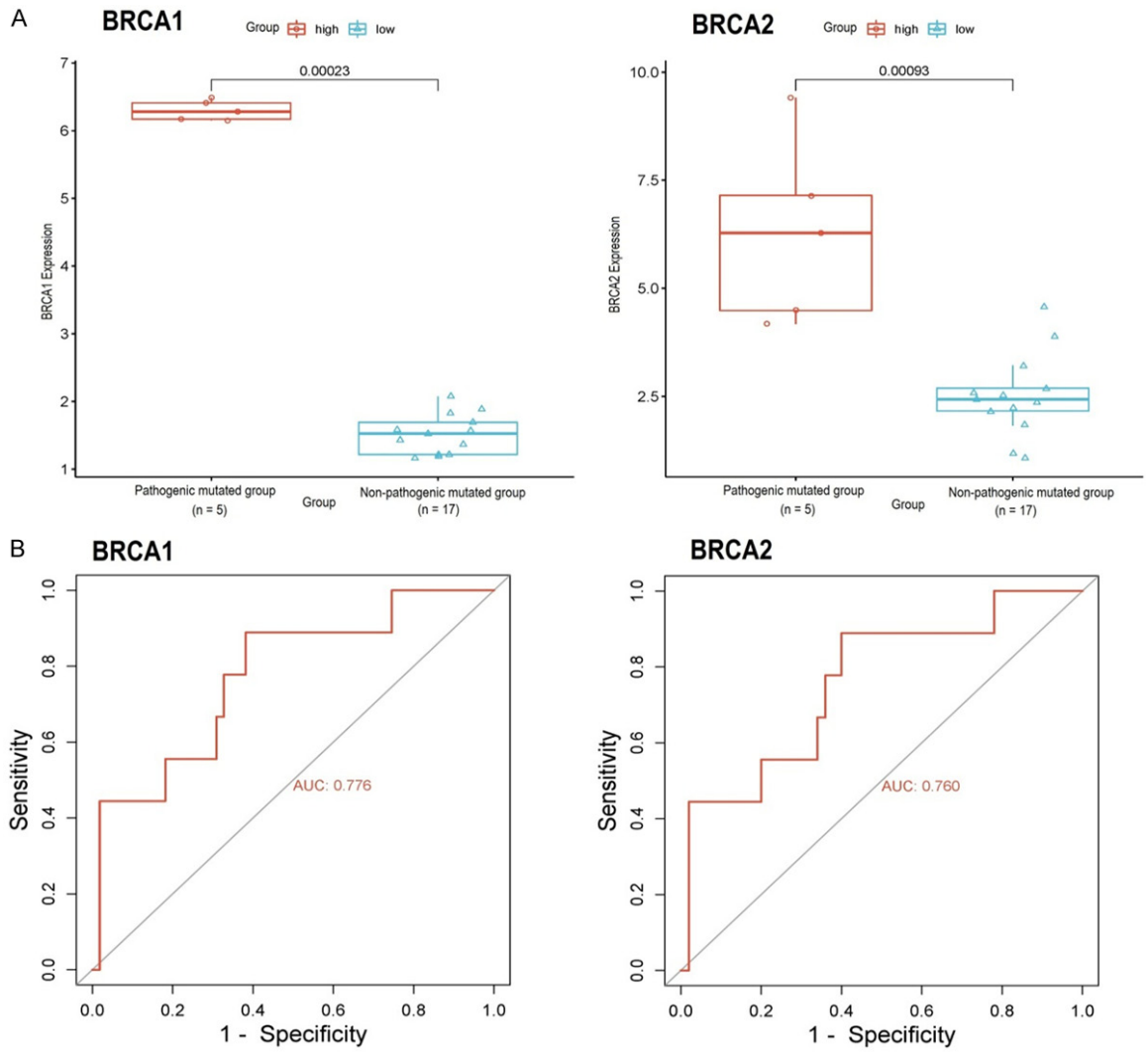

Figure 3. Relative expression and ROC curve analysis of BRCA1/2 genes between pathogenic mutated and non-pathogenic mutated HNSC sample groups. (A) Relative expression analysis of BRCA1/2 genes via RT-qPCR, and (B) RT-qPCR expression-based ROC curves of BRCA1/2 genes. A $P<0.05$ was used as the cut-off criterion.

Drug prediction analysis of BRCA1/2 genes

In this comprehensive investigation, we leveraged the DrugBank database to systematically explore therapeutic strategies aimed at modulating the expression of up-regulated and mutated BRCA1/2 genes. Our meticulous analysis revealed a diverse array of potential drug candidates, each showing promising characteristics for the regulation of BRCA1/2 expression. Notably, among these candidates, Arecoline, Estradiol, Bortezomib, Doxorubicin, Cyclosporine, Tretinoin, and Tamibarotene (**Table 3**) emerged as particularly significant. These compounds exhibit the potential to effectively down-regulate the expression levels of the BRCA1/2 genes, thus representing promising prospects for innovative therapeutic interventions.

Discussion

Head and neck squamous cell carcinoma (HNSC) remains a formidable challenge in oncology due to its aggressive nature and limited therapeutic options [40, 41]. Our study focused on the investigation of germline oncogenic mutations in the BRCA1 and BRCA2 genes among Pakistani HNSC patients and their potential clinical implications. We also explored the functional consequences of these mutations and their unique prevalence in this population.

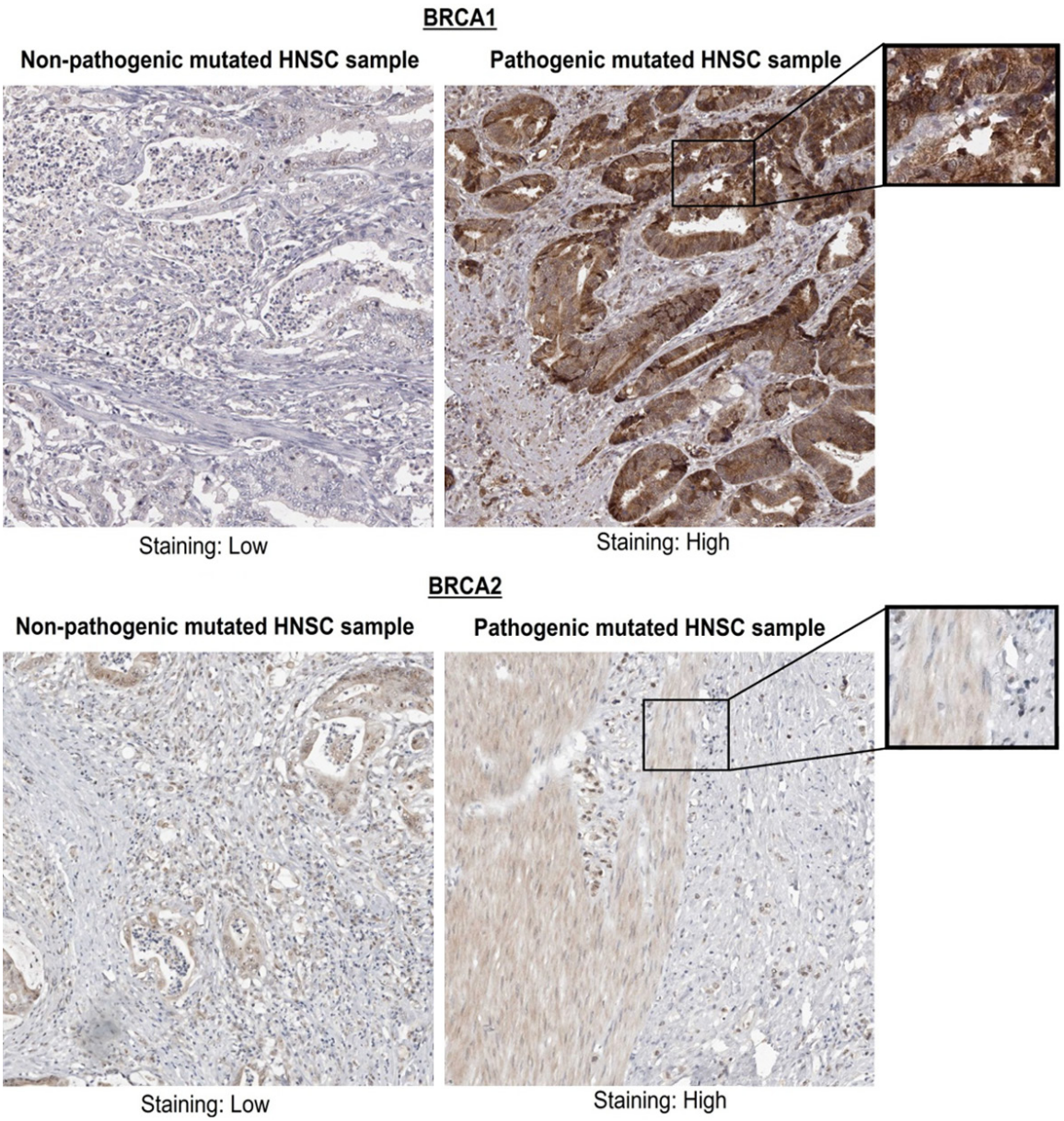

Figure 4. IHC-based proteomic expression analysis of BRCA1/2 proteins between pathogenic mutated and non-pathogenic mutated HNSC samples. Expression differences were measured based on the staining intensities.

One of the key findings of our study is the identification of three pathogenic mutations, including two in BRCA1 (p.Cys274Ter and p.Glu272Ter) and one in BRCA2 (p.Met1Val) genes among a group of Pakistani HNSC patients. Previously, the presence of pathogenic mutations in BRCA1 and BRCA2 genes has been associated with an increased risk of various cancers [42, 43], and recent studies have extended this association to HNSC [44-46]. The functional consequences of these mutations, including impaired DNA repair mechanisms, genomic instability, and susceptibility to carcinogenic insults, underscore their causative role in tumorigenesis [42, 47].

The identified 3 pathogenic mutations in our study are particularly significant because these mutations were not reported in HNSC patients in other populations, as confirmed by their absence in the gnomAD and TCGA HNSC dataset. These unique mutations suggest the existence of a distinct genetic profile in Pakistani HNSC patients, potentially contributing to their

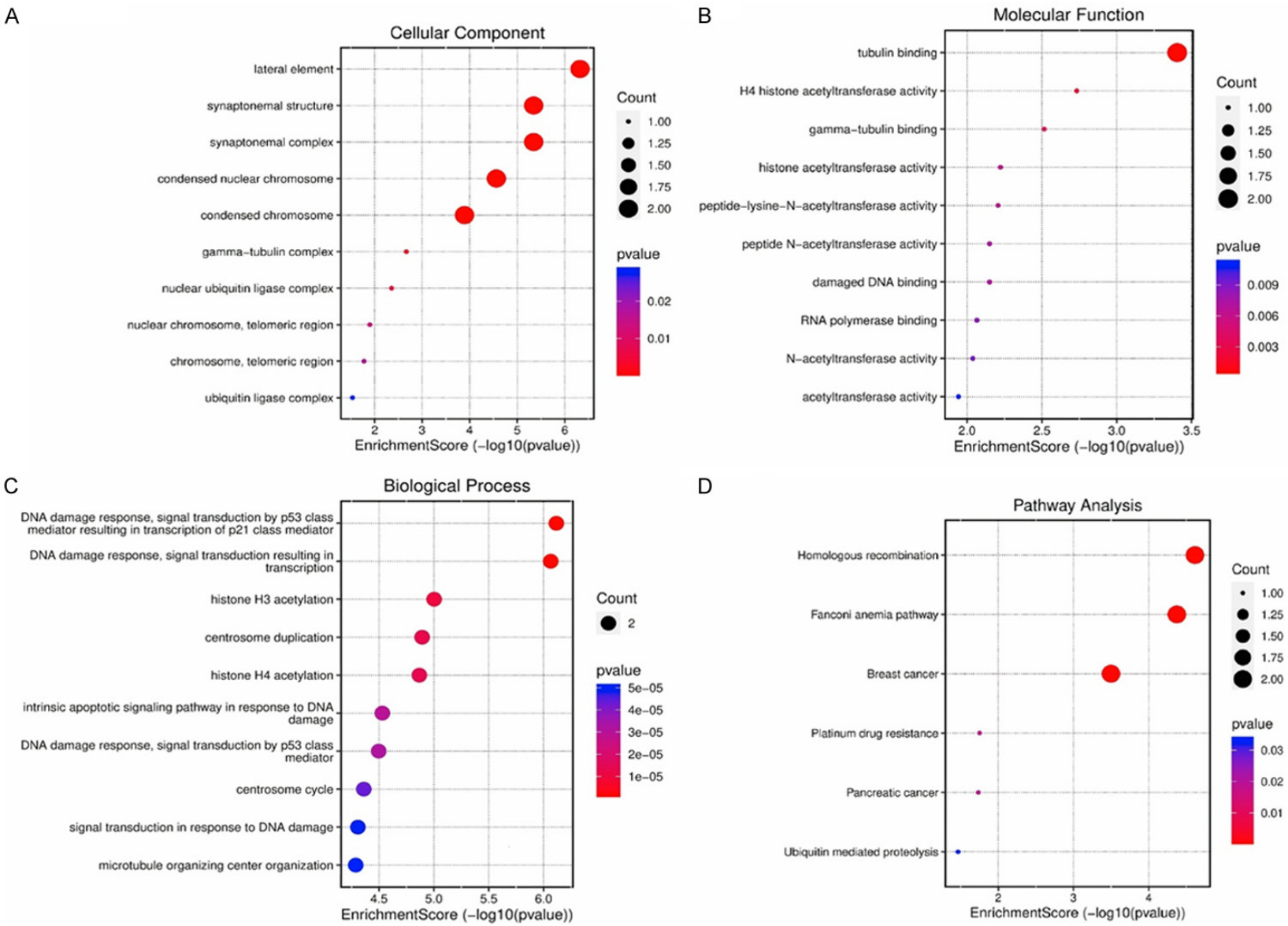

Figure 5. GO and KEGG analyses of BRCA1/2 genes via Metascape. (A) BRCA1/2 genes-related CC terms, (B) BRCA1/2 genes-related MF terms, (C) BRCA1/2 genes-related BP terms, and (D) BRCA1/2 genes-related KEGG terms. A $P<0.05$ was used as the cut-off criterion.

Table 3. DrugBank-based BRCA1/2 associated drugs

Sr. No	Hub gene	Drug name	Effect	Reference	Group
1	BRCA1	Arecoline	Decrease expression of BRCA1 mRNA	A20694	Approved
		Estradiol		A21155	
		Bortezomib		A21448	
		Cyclosporine		A20661	
2	BRCA2	Bortezomib	Decrease expression of BRCA2 mRNA	A21448	Approved
		Doxorubicin		A21498	
		Estradiol		A21155	
		Cyclosporine		A20661	
		Tretinoin		A24376	
		Tamibarotene		A24376	

susceptibility to this malignancy. The rarity of these pathogenic mutations in BRCA1 and BRCA2 genes within the global HNSC population underscores the importance of population-specific studies. These findings emphasize the necessity of tailoring diagnostic and therapeutic strategies to the genetic characteristics of specific populations, as genetic heterogeneity can significantly influence disease prevalence and outcomes.

Our study also explored the functional consequences of these pathogenic mutations by assessing the expression levels of BRCA1 and BRCA2 genes. Remarkably, we observed significantly higher expression levels of these genes in HNSC samples with pathogenic mutations compared to those without. Previously, it has been documented that pathogenic mutations in BRCA1/2 genes lead to the reduction in expression and the functional impairment of these genes. Consequently, these genes become less effective in repairing DNA damage, which results in the accumulation of mutations and abnormalities in the chromosomes [48-50]. In the present study, the up-regulation of BRCA1/2 genes due to pathogenic mutations may be indicative of an underlying mechanism contributing to tumor progression and aggressiveness in HNSC. Further investigations are warranted to delineate the precise molecular mechanisms driving this up-regulation of BRCA1/2 in HNSC.

Additionally, we conducted immunohistochemical (IHC) analysis to assess the protein expression of BRCA1 and BRCA2. Consistent with the gene expression findings, we observed higher staining intensities of BRCA1/2 proteins in HNSC samples harboring pathogenic mutations. This suggests that the observed mutations may influence the protein levels of BRCA1 and BRCA2, further supporting their potential role in HNSC development and progression.

Within the realm of KEGG pathways, BRCA1/2 genes exhibited significant enrichment in pathways related to “homologous recombination”, “the Fanconi anemia pathway”, “breast cancer”, “platinum drug resistance in cancer”, and more. The established role of these pathways in the development of cancer is widely recognized [51, 52].

Earlier, numerous therapeutic strategies have been explored to modulate BRCA1/2 gene expression. One approach involves using PARP inhibitors, which exploit the synthetic lethality concept in BRCA-deficient tumors [53, 54]. These inhibitors selectively target cancer cells with BRCA mutations, impairing their DNA repair capabilities and causing cell death. Additionally, gene therapy approaches aim to restore functional BRCA1/2 expression in mutated cells [55, 56]. However, challenges in efficient gene delivery and precise regulation persist. Epigenetic modulators, such as HDAC inhibitors, have also shown promise in down-regulating BRCA/2 genes [57].

In our quest to explore potential therapeutic avenues, we conducted a systematic analysis of drugs targeting the up-regulated and mutated BRCA1/2 genes. This analysis identified several promising drug candidates, including Arecoline, Estradiol, Bortezomib, Doxorubicin, Cyclosporine, Tretinoin, and Tamibarotene. These compounds hold substantial potential for innovative therapeutic interventions in HNSC, offering new avenues for treatment

modalities tailored to the genetic characteristics of Pakistani patients.

Conclusion

In conclusion, our study sheds light on the unique genetic landscape of HNSC in the Pakistani population, characterized by the presence of distinct pathogenic mutations in BRCA1 and BRCA2 genes. These mutations hold promise as valuable potential diagnostic biomarkers and therapeutic targets. Further research is essential to unravel the underlying molecular mechanisms and to translate these findings into clinical practice, ultimately improving the management and outcomes of HNSC patients in this specific population.

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Disclosure of conflict of interest

None.

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