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IDENTIFICATION OF MUTATIONS POTENTIALLY ASSOCIATED WITH CHEMORADIOTHERAPY TREATMENT RESPONSE IN CERVICAL CANCER

PATOS DE MINAS-MG
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Artigo Científico apresentado ao Instituto de Biotecnologia da Universidade Federal de Uberlândia como requisito final para a obtenção do título de Bacharel em Biotecnologia.

Prof. Dr. Matheus de Souza Gomes

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Os membros da Comissão Examinadora acima assinaram a Ata de Defesa que se encontra no Sistema Eletrônico de Informações (SEI) da Universidade Federal de Uberlândia.

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DEDICATÓRIA

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“We can judge our progress by the courage of our questions and the depth of our answers, our willingness to embrace what is true rather than what feels good”.

- Carl Sagan

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Identification of Mutations Potentially Associated with Chemoradiotherapy Treatment Response in Cervical Cancer

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ABSTRACT

Background

Chemoradiotherapy resistance can significantly influence treatment outcomes in cervical cancer patients. Somatic variants play a crucial role in tumor development and treatment response. This study aimed to identify potentially clinically significant variants associated with chemoradiotherapy response using RNA-seq data analysis.

Methods

RNA-seq data from 31 cervical cancer patients were subjected to variant calling and subsequent analysis using a machine learning decision tree algorithm. Variants within coding genes were selected based on their potential to segregate non-responder and

responder groups. The selected variants were further assessed using variant impact prediction tools.

Results

We identified and investigated variants that exhibited potential for distinguishing between non-responder and responder patients. Comprehensive analysis using variant impact prediction tools indicated that these variants may have deleterious effects on gene function. The genes harboring these variants were found to be involved in crucial cellular processes associated with cancer progression, including cell cycle regulation, proliferation, and notably, drug resistance.

Conclusions

Our findings suggest that specific variants, namely MUC6(NM_005961.3):c.5992C>A, NAP1L1(NM_004537.7):c.1064_1065delinsGG, PABPC1(NM_002568.4):c.1255C>T, and NLRP4(NM_134444.5):c.1488_1489insT, have the potential to serve as indicators of poor chemoradiotherapy response in cervical cancer patients.

Keywords

Cervical Cancer, RNA-seq, Chemoradiotherapy, Somatic Mutations, MUC6, NAP1L1, PABPC1, NLRP4.

Background

Cervical cancer (CC) continues to pose a significant global health burden, ranking as the fourth most prevalent cancer among women worldwide [1]. Locally advanced CC is typically treated with chemoradiotherapy (CRT), which is considered the standard therapeutic approach [2, 3]. CRT has shown a 5-year survival rate of approximately 70% and is associated with low rates of local and distant recurrences [4]. However, the efficacy

of CRT is closely intertwined with the development of radio-resistance and drug resistance, both of which are influenced by multiple cellular mechanisms, including proliferation, apoptosis, and cell cycle regulation [5].

Somatic mutations play a pivotal role in the development and progression of cancer by disrupting the cell cycle, promoting tumor growth, and contributing to treatment resistance [6]. Oncogenes and tumor suppressor genes are essential for maintaining critical cellular processes, including DNA repair, apoptosis, and cell cycle control [7]. Mutations in these genes can directly impair these processes, thereby promoting tumor formation. Additionally, somatic mutations can confer resistance to cancer treatments through various mechanisms. Mutations that affect drug targets or the drug metabolism pathway can render tumor cells resistant to chemotherapy, while the dysregulation of DNA repair pathways can impede treatment strategies reliant on inducing DNA damage [5].

The introduction of next-generation sequencing (NGS) has revolutionized cancer research and clinical practice, particularly in the field of RNA sequencing (RNA-seq). RNA-seq has emerged as a highly effective diagnostic tool, allowing for a comprehensive analysis of the transcriptome. By profiling gene expression patterns, RNA-seq provides valuable insights into the molecular landscape of cancer and aids in the identification of genetic biomarkers [8].

In addition to RNA-seq, computational tools have significantly enhanced the identification and analysis of genetic biomarkers associated with cancer. Machine learning algorithms, for example, can be leveraged to construct predictive models for classifying different cancer subtypes or predicting treatment response [9]. These

algorithms can integrate diverse genomic data, such as gene expression profiles, somatic mutations, and clinical variables, to generate robust and accurate predictions. Such approaches hold great promise in improving cancer diagnosis, treatment selection, and personalized therapy [9].

The integration of NGS data and computational tools into clinical decision-making processes offers significant potential. Genetic biomarkers identified through NGS can provide valuable information for treatment selection, prognosis assessment, and the development of personalized therapies [10]. By analyzing the genomic landscape of a patient's tumor, including somatic mutations, gene expression patterns, and other genomic alterations, NGS allows for a more precise and individualized approach to cancer treatment. To facilitate the translation of NGS data into clinical practice, concerted efforts are underway to establish standardized pipelines and databases. Initiatives like The Cancer Genome Atlas (TCGA) and the International Genome Consortium (ICGC) aim to compile comprehensive genomic data from various cancer types and make it accessible to researchers and clinicians [11]. These resources provide a wealth of information for analyzing and interpreting NGS data, enabling researchers and clinicians to make more informed decisions in the context of cancer diagnosis and treatment. Overall, the integration of NGS data and computational tools, along with the establishment of standardized pipelines and databases, holds great promise for advancing precision medicine and improving patient outcomes in cancer care.

The primary objective of this study was to gain deeper insights into the molecular landscape of CC and identify somatic mutations that hold potential as genetic biomarkers regarding CRT response. This was achieved through the analysis of RNA-seq data obtained from CC patients who underwent CRT. The discovery and characterization of

these specific mutations have the capacity to significantly augment the clinical decision-making process, facilitating the selection of personalized treatment strategies and ultimately leading to improved patient outcomes.

Materials and methods

Patients Recruitment and Samples Selection

A total of 31 CC patients were enrolled in this study at the Mario Penna Institute in Belo Horizonte, Brazil, between August 2017 and May 2019. The study protocol received ethical approval from the local Institutional Review Board (Approval No. 1.583.784). Inclusion criteria encompassed patients with no prior history of cancer or immune diseases, a histopathological diagnosis of cervical adenocarcinoma or squamous cell carcinoma, and a cervical cancer diagnosis classified as stage II or III according to the FIGO classification. Written informed consent was obtained from all participants. After diagnosis and biopsy, patients underwent CRT, and comprehensive clinical data were collected and analyzed. Patient classification was based on two primary criteria: treatment response (R) and non-response (NR). The determination of treatment response for each patient involved clinical examinations, pathological analyses, and imaging evaluations. Patients were categorized as R if no cervical neoplastic lesions were detected within 8 months post-chemoradiation. Conversely, patients were classified as NR if cervical neoplastic lesions persisted, including cases of partial response, tumor progression, or stable disease, up to 8 months after treatment.

Fluorescence-Activated Cell Sorting (FACS) and RNA sequencing

Tumor cell samples obtained from cervical biopsy underwent fluorescence-activated cell sorting (FACS) in order to differentiate cervical cancer stem-like cells (CCSCs) and non-stem cervical cancer cells (NSCCs) from a complex mixture of tumor cells based on their

light scatter and fluorescent staining profile. NSCCs were selected and processed using the SMART-Seq v4 Ultra-low Input RNA Sequencing Kit (Takara Bio USA, CA) following the manufacturer's instructions for cDNA synthesis. Subsequently, the RNA sequencing library was prepared using the Nextera® XT Library Prep Kit (Illumina™) along with the Nextera® XT Index Kit V2 Set A (Illumina™), as per the manufacturer's instructions. Detailed descriptions of these procedures were previously provided by our research group [12].

Transcriptome analysis

Our research group previously conducted the analysis of CCSCs [12]. For the analysis of NSCCs, the quality of the sequencing data was assessed using the FastQC tool [13]. Reads with low quality (Phred score ≤ 25) were filtered out, and adapters were trimmed using the Cutadapt tool [14]. After trimming, the remaining sequences underwent another round of quality assessment using FastQC to ensure the desired quality standards. Subsequently, the sequences were mapped and aligned to the human genome (Homo sapiens GRCh38) using the STAR software [15].

Variant calling, somatic filtering and variant data analysis

Variant calling was performed using BCFtools 1.16 [16]. In order to select high confidence variants, filtering was performed by requiring a minimum Phred quality score 30 and a read depth of 100 at the position. In order to exclude potentially germline variants, common population single nucleotide variations (SNVs) present in the dbSNP database were removed from the pool of detected variants.

The remaining variants were annotated using Ensembl Variant Effect Predictor (VEP) [17]. Vep includes population allele frequency from databases such as 1000 Genomes

Project and Aggregation Database (gnomAD). Variants with population frequencies of 1% or higher were filtered out to further remove germline variants. Multiple impact prediction tools available on VEP were employed, including SIFT, PolyPhen-2, CADD and LOUEF. SIFT Indel [18] and SpliceAI-visual [19] were employed to assess the effects of indels and splice region variants respectively.

Vcf2maf [20] was used to generate MAF files from the final pool of variants for further analysis. Maftools and GenVisR R packages were used for data processing and visualization.

Association of variants to treatment response

Using the full training approach, decision tree analysis was conducted on somatic variants of coding genes. The analysis was performed using the WEKA software (Waikato Environment for Knowledge Analysis, version 3.6.11, University of Waikato, New Zealand) [21].

Results

Cohort Clinical Description

Samples from 31 women diagnosed with CC were subjected to NGS analysis. Patient clinical information and histopathological analysis are summarized in Table 1. Overall response was evaluated 8 months after ending treatment, 21 patients were classified as Responders (R) and 10 as Non-responders (NR).

Table 1: Clinicopathological characteristics and chemoradiotherapy response.

Patient characteristics		N	Value
Age	Years	31	52.3±16.8
Diagnostic	SCC	30	96.8
	ICA	1	3.2
	IIA	1	3.2

FIGO Stage	IIB	15	48.4
	IIIB	15	48.4
Histological grade	II	13	41.9
	III	13	41.9
	NA	5	16.1
Parametrial involvement	Free	1	3.2
	Unilaterally	7	22.6
	Bilaterally	22	71.0
	NA	1	3.2
Vaginal involvement	Yes	28	90.3
	No	1	3.2
	NA	2	6.5
Lymph node status	NX	8	25.8
	N0	9	29.0
	N1	14	45.2
Metastasis	MX	10	32.3
	M0	9	29.0
	M1	12	38.7
Tumor size (cm)	<4	1	3.2
	≥4	29	93.5
	NA	1	3.2
Overall response	R	21	67.7
	NR	10	32.3

The values represent the mean + standard error or %. SCC, Squamous Cell Carcinoma; ADC, Adenocarcinoma; NR, Non-Responders, R, Responders; NX, Regional lymph nodes cannot be assessed; N0, No regional lymph node metastasis; N1, Regional lymph node metastasis; MX, Distant metastasis cannot be assessed; M0, No distant metastasis; M1, Distant metastasis. NA, Not Available.

Cohort Mutation Landscape

In total, 34,617 unique variants were found within the cohort, comprised of 30,851 (89.12%) base substitutions and 3,766 (10.88%) indels. 24,432 (79.58%) variants were classified as novel, while 10,185 (29.42%) were previously annotated in variant databases. Only 522 (1.51%) variants were located in coding genes, the vast majority of the identified variants were located in non-coding transcripts of varying types, with long non-coding RNA being the most predominant. Only coding gene variants were selected for further analysis in this study, and a comprehensive statistical summary of this data is available in Supplementary Figure 1.

In the top 10 mutated genes, we observed the recurrence of genes MUC3A, MUC6, HLA-DRB1 and GOLGA6L10 [22,23,24], which were previously reported in CC, and novel genes SPATA22, FER1L5, SPDYE1, ACTR3B, ADAMTSL3 and TRIM45. Figure 1 displays an overview of the most mutated genes per patient, including the presence of variants in non-coding regions.

Variants associated to non-response

A total of 522 coding gene variants were included in the decision tree analysis. The decision tree successfully segregated responders and non-responder patients based on four junction points, each corresponding to a different variant (Figure 2).

These variants were labeled as NLRP4(NM_134444.5):c.1488_1489insT MUC6(NM_005961.3):c.5992C>A, PABPC1(NM_002568.4):c.1255C>T, and NAP1L1(NM_004537.7):c.1064A>G. It is worth noting that although the decision tree only reported one variant per gene, further investigation showed the co-occurrence of two additional SNVs in the NAP1L1 gene. One was labeled as NAP1L1(NM_004537.7):c.1062T>C, while the other was adjacent to NAP1L1(NM_004537.7):c.1064A>G, revealing them to be a deletion-insertion (delins) which we labeled NAP1L1(NM_004537.7):c.1064_1065delinsGG. The additional variants have been included in our analysis in order to provide a more comprehensive overview of the genetic variations within that gene. A comprehensive summary report of the variants, including known functions of affected genes, is available in Table 2. The deleterious potential of missense variants was assessed using scores from different variant impact prediction tools available on VEP (Table 3). Prediction tools SIFT Indel and SpliceAI were employed on suitable variants.

Table 2: Summary of variants identified as potential indicators of poor CRT response

Variant	Consequence	Patients	Database IDs	Population frequency	Gene functions
MUC6(NM_005961.3):c.5992C>A	Missense	17, 48, 123	rs756062369 COSV70132617 COSV70143795	0.00000798	Extracellular matrix structural constituent, maintenance of gastrointestinal epithelium, β-catenin degradation
NAP1L1(NM_004537.7):c.1064_1065delinsGG	Missense	63, 97	novel	Not found	Histone chaperone, nucleossome assembly, nucleotide excision repair, mitotic DNA double strand break repair, cell cycle progression
NAP1L1(NM_004537.7):c.1062T>C	Synonymous & Splice region		novel	Not found	
PABPC1(ENST00000318607.10):c.1255C>T	Missense	17, 63	rs766099049	0.00428	mRNA Translation, mRNA Decay, miRNA-mediated repression, NMD, L1 Retrotransposition, mRNA localization, local translation
NLRP4(NM_134444.5):c.1488_1489insT	Frameshift insertion	103, 121	novel	Not found	Inflamatory response, Regulation of inflamatory response, NF-kB inhibition

Table 3: Missense variants and respective assigned scores from variant impact prediction tools.

Variant	SIFT	PolyPhen-2	BLOSUM62	CADD	LOEUF	PhyloP100way
MUC6(NM_005961.3):c.5992C>A	tolerated(0.12)	probably_damaging (0.912)	-1	8.197	0.97	-1.725
NAP1L1(NM_004537.7):c.1064_1065delinsGG	tolerated(0.11)	probably_damaging (0.999)	-1	#	#	8.517*
						5.574**
PABPC1(NM_002568.4):c.1255C>T	deleterious(0.03)	possibly_damaging (0.865)	-3	26.3	0.27	6.313

Values considered to deem each parameter as evidence of deleteriousness were: SIFT < 0.05; PolyPhen > 0.85; CADD ≥ 20; LOUEF < 0.35; BLOSUM62 < 0; phyloP100way ≥ 8.0. Conservation scores in NAP1L1(NM_004537.7):c.1064_1065delinsGG were calculated for each individual base affected at postions 1065* and 1064**.

MUC6(NM_005961.3):c.5992C>A

MUC6 emerged as a highly mutated gene in multiple patients within the cohort (Supplementary figure 2A). Six unique variants were identified in this gene. Apart from the reported variant, patients 48 and 123 harbored two other missense variants in MUC6, and patient 17 harbored three. The reported variant showed evidences of having functional impact on the protein, but the consensus from multiple prediction tools and

conservation analyses would provide a more comprehensive assessment of its significance.

NAP1L1(NM_004537.7):c.1064_1065delinsGG and NAP1L1(NM_004537.7):c.1062T>C

NAP1L1 was another highly mutated gene in our cohort (Supplementary Figure 2B), displaying nine unique variants. On patient 63, the reported variants were the only ones present in NAP1L1, while patient 97 exhibited seven other variants within the gene. The delins variant was not evaluated with as much scrutiny as the others due to some prediction tools not supporting delins data input, nonetheless, amino acid substitution and conservation scores indicated it as potentially damaging to protein structure. NAP1L1(NM_004537.7):c.1062T>C is a synonymous mutation located on the splice region of an exon, however, results from SpliceAI indicate it has no effect on splicing (Supplementary Figure 3).

PABPC1(NM_002568.4):c.1255C>T

PABPC1 exhibited a moderate level of mutation in a few samples within the cohort (Supplementary Figure 2C). The reported variant achieved a consensus among most prediction scores as potentially damaging to the protein product. Interestingly, patient 63 harbored a nonsense mutation (PABPC1(NM_002568.4):c.874C>T) that also appeared in patient 108, a responder. The presence of a nonsense mutation in PABPC1 suggests potential disruption of protein function and highlights its possible involvement in treatment response or disease progression.

NLRP4(NM_134444.5):c.1488_1489insT

In contrast to the other genes analyzed, NLRP4 did not exhibit any additional mutations beyond the one reported by the decision tree (Supplementary Figure 2D). The identified variant in NLRP4 is a frameshift insertion involving a single nucleotide. This variant has been classified as deleterious by the SIFT Indel prediction tool with a confidence score of 0.858. The frameshift insertion in NLRP4 has a significant impact on the protein structure and function. It results in a disruption of almost half of the amino acid sequence, which can lead to profound changes in protein folding, interaction with other molecules, and overall protein function. Furthermore, according to SIFT Indel the variant is predicted to trigger nonsense-mediated decay, a cellular mechanism that targets mRNA with premature termination codons for degradation. These findings suggest that the frameshift insertion in NLRP4 is likely to have significant functional consequences and may contribute to poor CRT response or CC characteristics within the cohort.

Discussion

With the advancements in next-generation sequencing technologies, RNA-seq has emerged as a popular and cost-effective tool for studying gene expression and providing valuable insights into the phenotype. Despite its widespread use, accurately identifying variants from RNA-seq data can be challenging due to its inherent limitations, such as lower accuracy compared to DNA sequencing. However, several studies have successfully employed rigorous filtering criteria and other strategies to obtain reliable variant calling results from RNA-seq data [25, 26]. Considering these factors, in our study, we performed variant calling, followed by stringent filtering and processing steps, in order to map out the mutational landscape of the cohort. This analysis served two primary purposes. Firstly, it aimed to provide a comprehensive overview of the mutational profile of each patient within the cohort, shedding light on the specific genetic

alterations present in CC. Secondly, by comparing our findings to previously validated CC variant data from other studies, we aimed to assess the accuracy and reliability of our variant calling pipeline. This approach allowed us to gain valuable insights into the mutational landscape of CC in our cohort and evaluate the robustness of our variant calling methodology. By corroborating our findings with validated data from other studies, we aimed to ensure the accuracy and reliability of our results, thus strengthening the scientific foundation of our research and contributing to the growing body of knowledge in the field of CC genomics.

In our cohort, we observed the presence of several genes that belong to families frequently associated with CC development, including HLA genes and mucins. The HLA region genes play a crucial role in the immune response against tumor antigens, highlighting their significance in CC [27]. Specifically, HLA-A and HLA-B have been identified as driver genes in CC [22], and mutations in HLA-DRB1 have been associated with the development of this type of cancer [28]. Mucins, on the other hand, are known to participate in inflammatory responses, and their dysregulation is often associated with tumor progression. However, it is important to note that some mucins have been reported to act as tumor suppressors in various cancers, such as MUC2 and MUC6 [29]. In our cohort, we identified frequent mutations in MUC6 and MUC12, which have been previously reported in cervical carcinomas [24]. Additionally, MUC3A has displayed tumorigenesis potential in cervical adenocarcinoma [30]. Furthermore, we also detected other genes commonly found to be associated with CC. These findings support the involvement of these genes in CC development and emphasize their potential relevance as therapeutic targets or biomarkers for this disease. By identifying these genes within our cohort, we contribute to the growing understanding of the molecular landscape of CC

and provide valuable insights into the potential mechanisms driving its development and progression. Further investigations are warranted to explore the functional implications of these genetic alterations and their clinical significance in CC.

Through the implementation of a decision tree-based machine-learning algorithm, we successfully identified specific variants that have the potential to impact response in CRT treatment. To ascertain the significance of these variants, we conducted a comprehensive analysis considering various factors. Firstly, we assessed the potential deleterious effects of the variants by examining factors such as amino acid substitution, nucleotide conservation, and population frequencies. These criteria allowed us to gauge the likelihood of these variants causing functional alterations at the protein level and their relative rarity within the population. Furthermore, we delved into the functional characteristics of the affected genes to ascertain their involvement in cellular mechanisms associated with CRT response. This evaluation provided insights into the potential interactions between these genes and the relevant molecular pathways underlying treatment outcomes. By integrating these analyses, we aimed to establish a comprehensive understanding of the potential impact of the identified variants on CRT response. This information is crucial for guiding further investigations and developing personalized treatment approaches in order to enhance patient outcomes and optimize therapy strategies for CC.

MUC6, although commonly used as an immunohistochemical marker to differentiate subtypes of endocervical carcinomas [31], its specific role in CC remains relatively unexplored. However, its tumor suppressor role has been well-documented in other cancer types such as pancreatic cancer, renal cancer, gastric cancer, and colorectal cancer [32-35]. Studies have shown that MUC6 acts as a tumor suppressor by inducing β-catenin

degradation through the autophagy pathway [33]. β-catenin plays a crucial role in regulating cell growth and intracellular adhesion in epithelial cells. In CC therapy, targeting β-catenin has gained considerable attention due to its involvement in cell proliferation and its contribution to chemotherapy and radiotherapy resistance [36]. Understanding the functional significance of MUC6 in CC could provide valuable insights into its potential role as a therapeutic target or prognostic marker. By elucidating the molecular mechanisms by which MUC6 influences cellular processes and affects the behavior of CC cells, we can potentially identify new avenues for personalized treatment approaches and develop strategies to overcome therapeutic resistance.

NAP1L1, a nucleosome assembly protein, plays a crucial role in a diverse range of cellular functions. It has been implicated in regulating DNA replication, chromatin formation, cell cycle progression, and proliferation, among others [37]. Although its association with CC has not yet been established, multiple studies have demonstrated its involvement as a promoter of tumor cell proliferation in various cancer types [38-40]. Furthermore, there is evidence suggesting that NAP1L1 may contribute to chemotherapy resistance [41]. Understanding the precise role of NAP1L1 in CC could shed light on its potential as a therapeutic target and provide insights into mechanisms underlying tumor progression and treatment response. Given the multifaceted nature of NAP1L1's cellular functions, further investigation is warranted to elucidate its specific involvement in CC development and progression. This knowledge could pave the way for the development of targeted therapies aimed at modulating NAP1L1's activity and potentially overcoming chemoresistance in CC treatment.

PABPC1, a highly conserved poly A-binding protein, is involved in regulating various aspects of mRNA metabolism, thereby connecting it to a wide range of cellular processes

[42]. In the context of CC, PABPC1 has been found to play a role in the inhibition of the Wnt/β-catenin pathway. A study uncovered the involvement of the long non-coding RNA (lncRNA) PDHB-AS as a tumor suppressor in CC. PDHB-AS was found to recruit PABPC1, leading to the inhibition of nuclear import of β-catenin and subsequent inactivation of the Wnt/β-catenin pathway [43]. The same study revealed that PDHB-AS is downregulated in CC, suggesting a potential role in disease progression. Additionally, the overexpression of PDHB-AS was shown to reduce cisplatin resistance, indicating its potential as a therapeutic target to enhance treatment response in CC [43]. The findings highlight the significance of PABPC1 in modulating the Wnt/β-catenin pathway and its potential implication in CC development and treatment resistance.

NLRP4 is a gene involved in the regulation of inflammatory response and functions as a negative regulator of pyroptosis, a form of programmed cell death triggered by pro-inflammatory signals [44]. Pyroptosis, despite causing cell death, has a dual role in CC progression due to its impact on the tumor microenvironment. In early stages of CC, pyroptosis acts as a suppressor by eliminating tumor cells. However, in advanced stages, tumor cells can exploit the tumor microenvironment, including the release of inflammasomes and pro-inflammatory molecules associated with pyroptosis, to promote invasion and metastasis [45, 46]. The intricate relationship between NLRP4, pyroptosis, and CC suggests the potential involvement of inflammatory pathways in tumor progression and metastasis.

The decision tree analysis conducted in our study identified specific variants that demonstrated potential for distinguishing between non-responder and responder groups. The genes where these variants are located have been extensively associated with various types of cancer, and their mechanisms have been well-documented. Based on the

predictions of variant impact prediction tools, we found evidence that four out of these mutations are likely to have deleterious effects on their associated genes: MUC6(NM_005961.3):c.5992C>A, NAP1L1(NM_004537.7):c.1064_1065delinsGG, PABPC1(NM_002568.4):c.1255C>T and NLRP4(NM_134444.5):c.1488_1489insT. These variants suggest a potential role in hindering CRT response. However, it is important to note that further research is necessary to validate and confirm these findings. Expanding the sample size and including a larger cohort of patients would enable us to determine the exclusivity of these variants to non-responder patients. Such data could then be utilized in the decision-making process for selecting appropriate treatment strategies for CC patients. Conducting additional investigations and studies will enhance our understanding of the clinical significance of these variants and their potential implications for treatment outcomes in CC.

Conclusions

CC is one of the most common gynecological cancers and has a high lethality rate, therefore, choice of treatment is a crucial decision in ensuring the health and well-being of CC patients. Despite being the recommended form of treatment in locally advanced CC, poor CRT response remains an obstacle to be overcome. Our data suggests that the somatic variants MUC6(NM_005961.3):c.5992C>A, NAP1L1(NM_004537.7):c.1064_1065delinsGG, PABPC1(NM_002568.4):c.1255C>T and NLRP4(NM_134444.5):c.1488_1489insT may be potential indicators of non-response to CRT. Further research is required in order to validate these findings and to explore their biological and clinical implications. Precise understanding of the interactions between these variants and CC may contribute to guiding treatment selection for CC patients.

List of abbreviations

CC: Cervical cancer

CRT: Chemoradiotherapy

NGS: Next generation sequencing

RNA-seq: RNA sequencing

R: Responder

NR: Non-responder

CCSC: Cervical cancer stem-like cells

NSCCs: Non-stem cervical cancer cells

VEP: Variant Effect Predictor

SNV: Single nucleotide variation

delins: Deletion-insertion

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Declarations

Ethics approval and consent to participate

The studies involving human participants were reviewed and approved by Mario Penna Institutional Review Board #1.583.784. The patients/participants provided their written informed consent to participate in this study.

Consent for publication

Not applicable.

Availability of data and materials

The data used in this article is available at the NCBI's BioProject site under the accession number: PRJNA705088.

Competing interests

The authors declare that they have no competing interests.

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Author's contributions

ACFF accomplished the bioinformatic analyses and drafted the manuscript; TCA and MSG participated in the study design and writing of the manuscript; ABMN contributed to the bioinformatics analyses; LRA and PLLB contributed to data processing *via* machine learning; MSG coordinated the project.

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Anexos

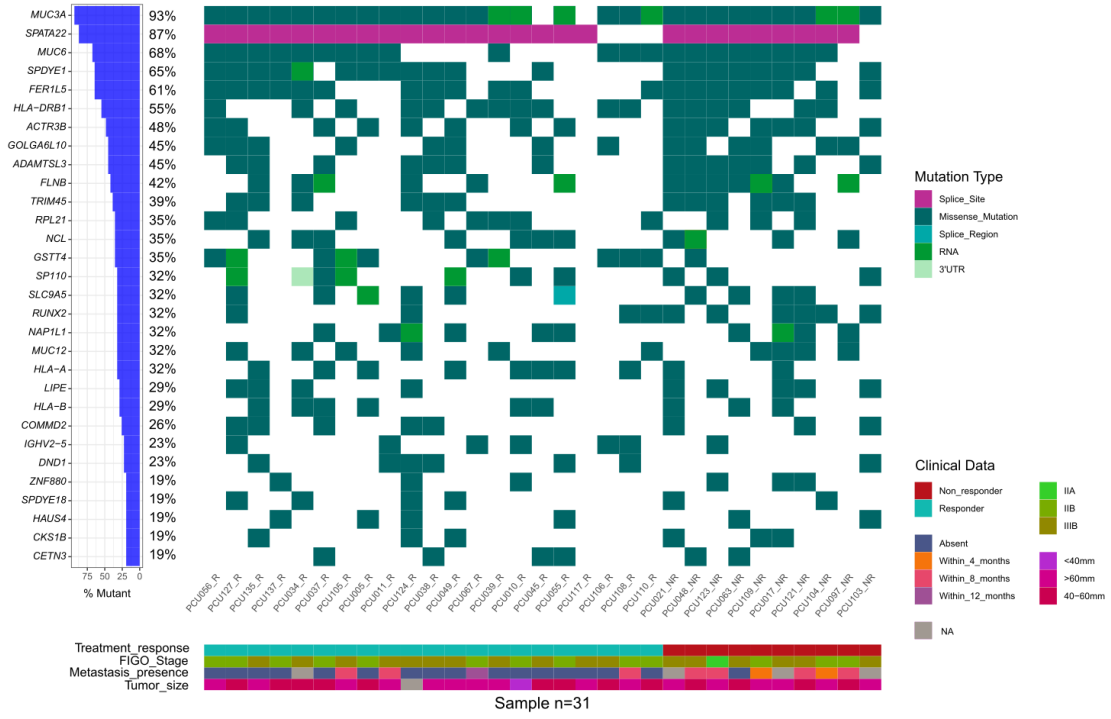


Figure 1: Distribution of frequently mutated genes in each patient. The bar plot on the left represents the percentage of patients harboring mutations in each gene. Patient clinical

data is displayed under their respective columns. RNA mutation type refers to variants located in non-coding transcripts from that gene. If multiple mutations were found in a gene in a single sample, only one is shown in the order as listed in the color legend from top to bottom.

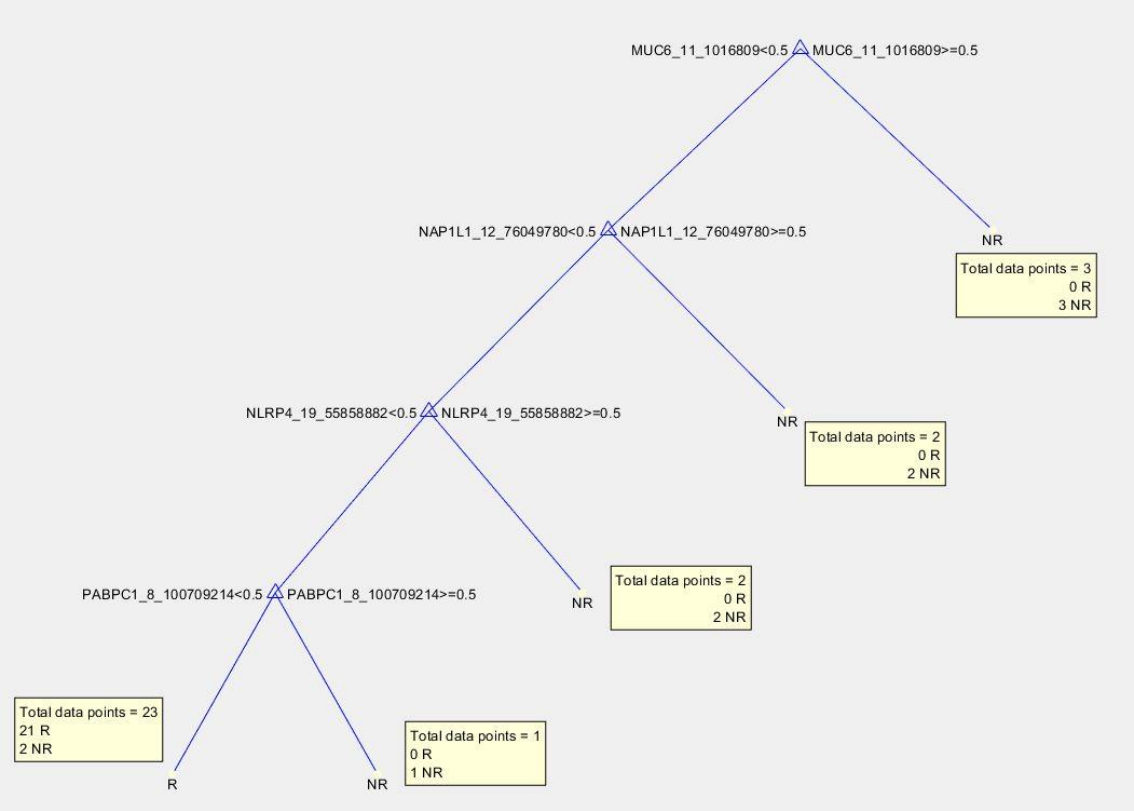
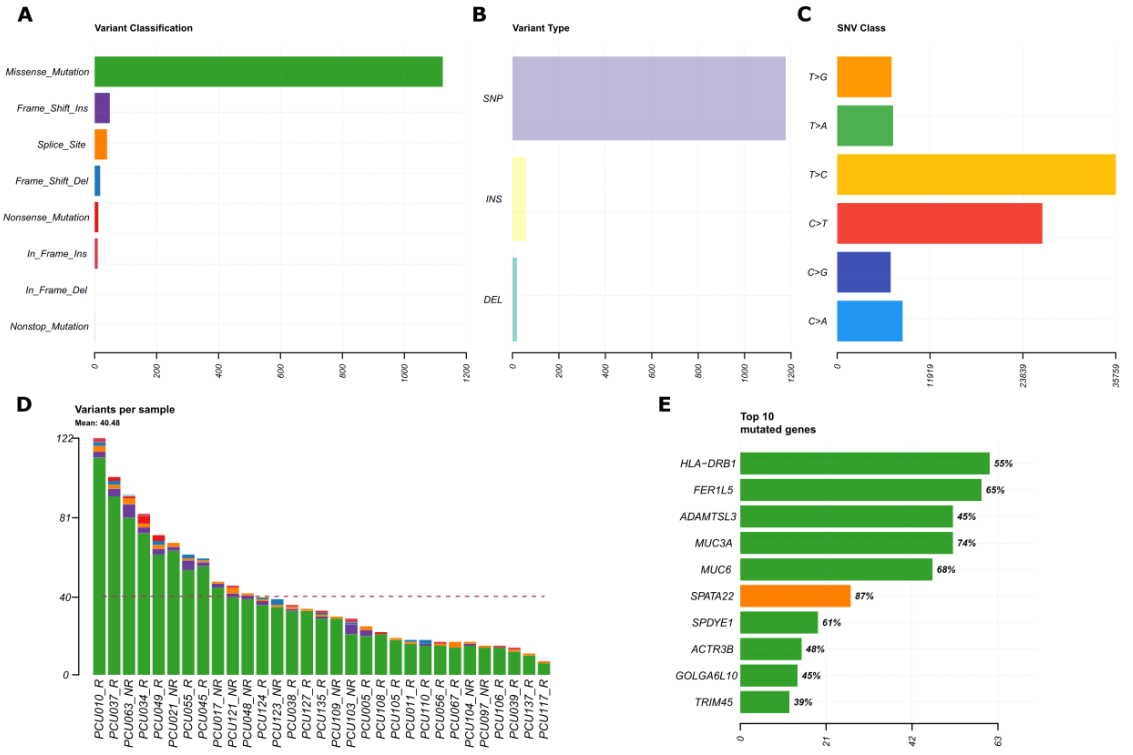
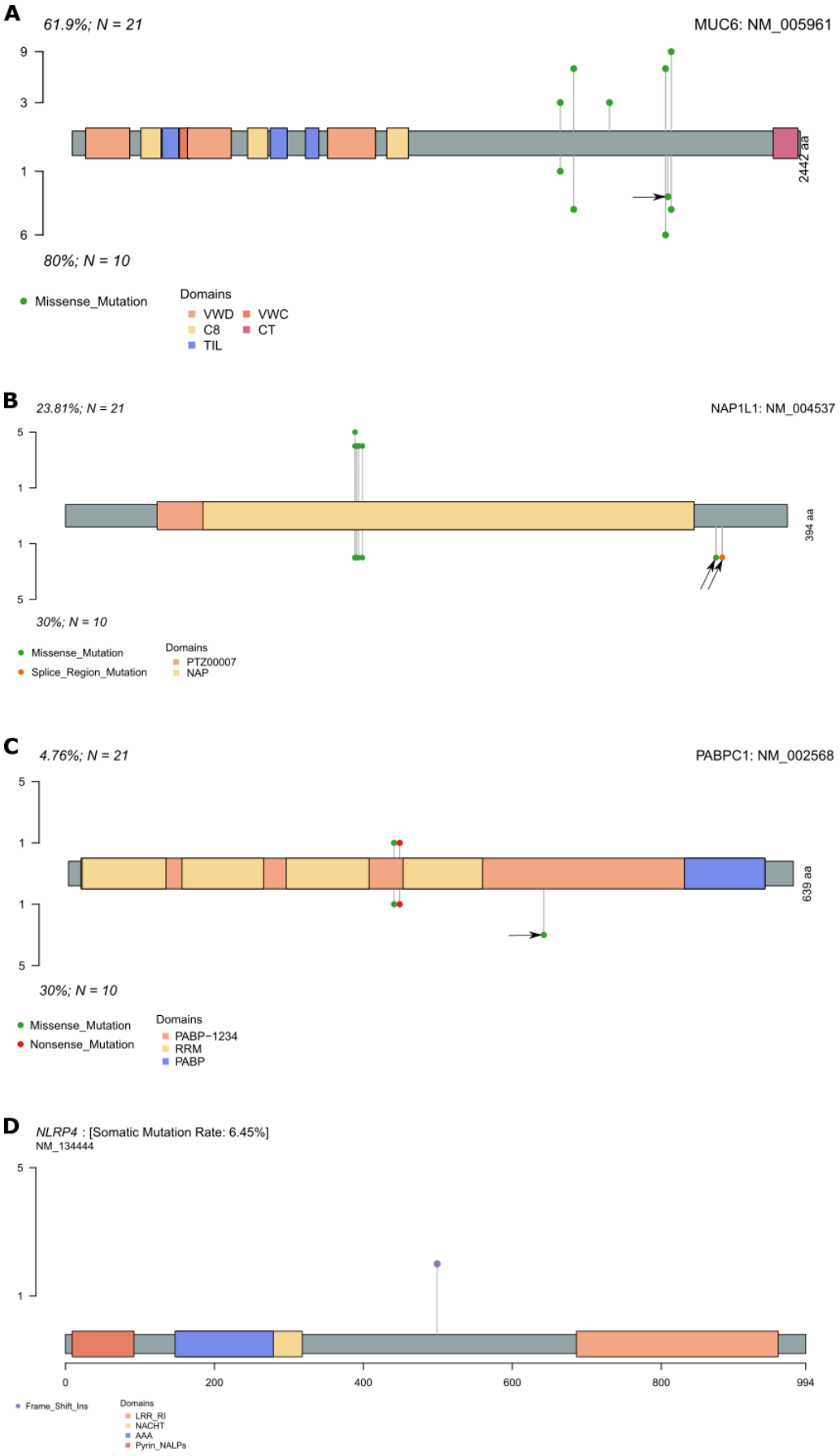


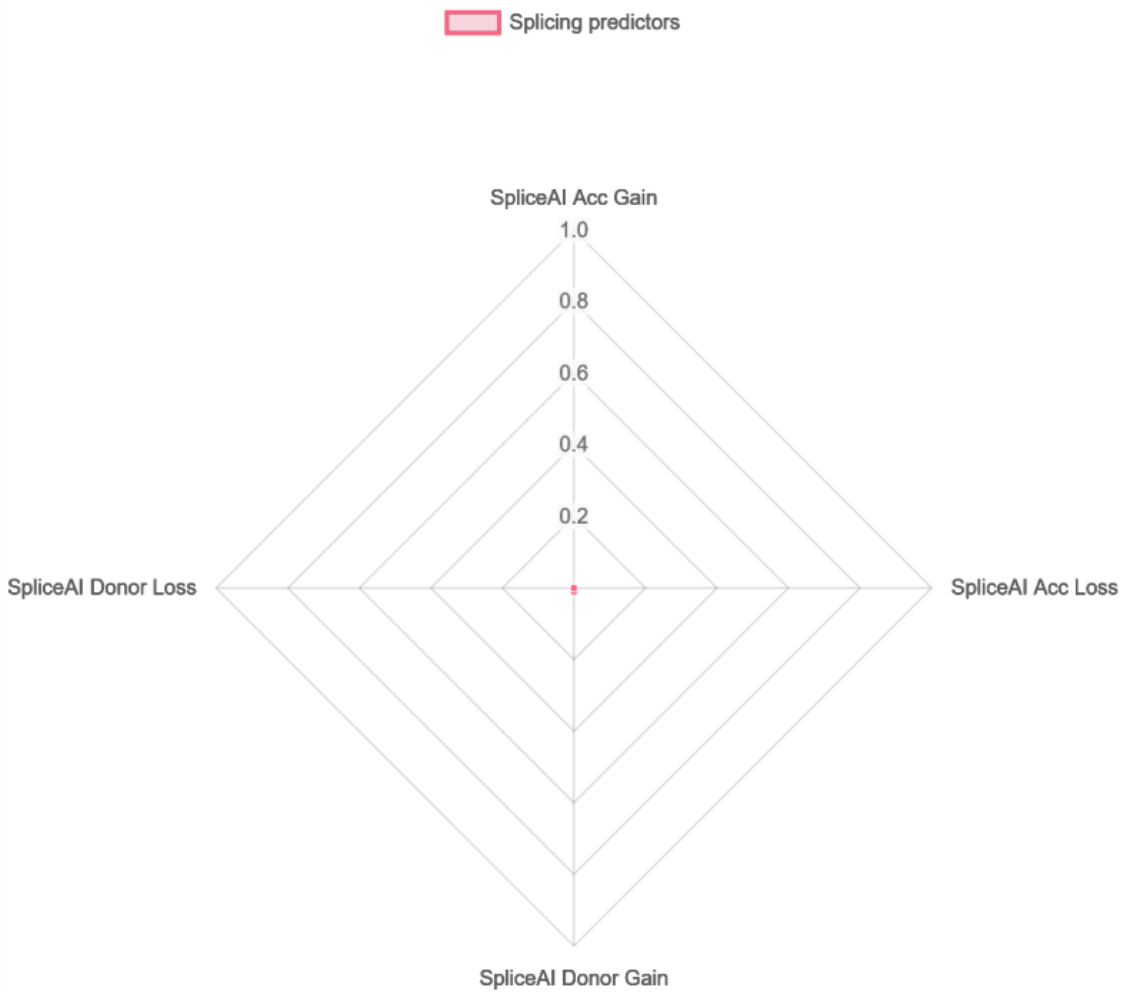
Figure 2: Decision Tree representation of the classification model using full training approach and variants related to non-response as attributes. The tree structure illustrates the hierarchical decision-making process based on key features, leading to the classification of variants into distinct categories.



Supplementary Figure 1: Summary of variant data from 31 patients. (A) Variant classification displaying total number of mutations identifies of each kind; (B) Number of SNPs, insertions and deletions indentified; (C) Distribution of base substitutions among SNPs; (D) Number of coding gene variants per sample; (E) Top 10 mutated genes. D and E follow the same color scheme presented in A to differentiate variant classes.


A
61.9%; N = 21
MUC6: NM_005961
9
3
1
6
2442 aa
80%; N = 10
Missense_Mutation
Domains
VWD
VWC
C8
CT
TIL
B
23.81%; N = 21
NAP1L1: NM_004537
5
1
1
5
394 aa
30%; N = 10
Missense_Mutation
Splice_Region_Mutation
Domains
PTZ00007
NAP
C
4.76%; N = 21
PABPC1: NM_002568
5
1
1
5
639 aa
30%; N = 10
Missense_Mutation
Nonsense_Mutation
Domains
PABP−1234
RRM
PABP
D
NLRP4 : [Somatic Mutation Rate: 6.45%]
NM_134444
5
1
0
200
400
600
800
994
Frame_Shift_Ins
Domains
LRR_RI
NACHT
AAA
Pyrin_NALPs

Supplementary Figure 2: Mutations identified across the protein structures of MUC6(A), NAP1L1(B), PABPC1(C) and NLRP4(D) proteins. Arrows indicate the variants from **Table 1** when multiple variants are located on the same gene. Percentages relate to number of R (N = 21) and NR (N = 10) patients harboring mutations in the gene. In A, B and C, where both R and NR patients harbored mutations in those genes, the top side represents mutations in R patients, while the underside represents NR patients.



Supplementary Figure 3: Splicing radar displaying predicted impact from NAP1L1(NM_004537.7):c.1062T>C.