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Original Article

Molecular Analysis of Mutations in the PD-L1 and PD-1 Genes in Squamous Cell Carcinoma of the Cervix

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Abstract

Background: Understanding the type, frequency, and location of mutations in the programmed death receptor-1 ligand (PD-L1) and programmed death receptor-1 (PD-1) genes in cervical cancer is essential for advancing our knowledge of the underlying mechanisms of gene mutation in the disease. This understanding is crucial for developing targeted therapies, improving early detection techniques, and providing individualized treatment plans for those affected by cervical cancer. This study aimed to determine mutations in PD-L1 and PD-1 genes in Squamous Cell Carcinoma of the Cervix.

Methods: This retrospective study employed a total of twenty formalin-fixed, paraffin-embedded tissue blocks of cervical cancer. The Nucleic Acid Amplification Technique was used, including DNA extraction and Polymerase Chain Reaction for DNA sequencing. The amplified fragments were sequenced using a genetic analyzer 3130xl sequencer. Kapelan Bio-Imaging Solutions software version 2.7.2 was used to measure the length of the PD-L1 and PD-1 isoforms. Data were presented as simple frequency and percentage using an Excel spreadsheet.

Results: The photomicrograph of the benign cervical cancer showed rounded to oval structures with layers of stratified squamous epithelial cells with distinct nuclei and no signs of dysplasia. In contrast, the photomicrograph of the malignant cervical cancer tissue showed dysplasia, nuclear polymorphism, increased nucleo-cytoplasmic ratio, and disorganized tissue architecture. Single nucleotide polymorphisms (SNPs) and functional mutation occurred along PD-L1 and PD1 genes respectively. Transversion mutation occurred in 37.5% of SNPs along the PD-L1 gene, while transition mutation occurred in 62.5% of SNPs along the PD-L1 gene. Missense mutation recorded 100% occurrence in the PD-L1 gene, while the PD-1 gene had 80% silent mutation and 20% missense mutation respectively.

Conclusion: Mutations were found in both PD-L1 and PD-1 genes in cervical cancer cells, with a greater number of mutations in the PD-L1 gene compared to the PD-1 gene, providing potential therapeutic

targets for PD-1/PD-L1 inhibitors. As PD-1/PD-L1 blockade therapy is expected to become a prominent cancer immunotherapy technique soon, targeting PD-1 or PD-L1 in cancer immunotherapy may effectively induce long-lasting anticancer immune responses with reduced toxicity in various cancers, particularly cervical cancer.

Keywords: Cervical cancer, PD-L1, PD-1, squamous cell carcinoma, human papilloma virus

Introduction

Cervical cancer is the fourth most common malignant tumor and the primary cause of cancer-related death in women, particularly in areas with relatively low human development indices. These indices include life expectancy, income, and level of education (1). Approximately 90% of deaths from cervical cancer occur in low- and middle-income countries. In Nigeria, cervical cancer ranks as the second most common cancer among women aged 15 to 44 years and the third most common overall (2). While the implementation of cancer screening and the widespread use of human papillomavirus (HPV) vaccines have the potential to reduce the incidence and mortality of cervical cancer, 15% of patients are diagnosed with metastatic disease at the time of diagnosis (3). The only available treatment for patients with advanced or metastatic cervical cancer is chemoradiotherapy with or without anti-angiogenesis agents, but the survival rate remains relatively low (4).

Patients with early-stage cervical cancer can benefit from radical surgical resection or radiation therapy, resulting in a favorable 5-year overall survival (OS). Those undergoing surgical resection for early-stage cervical cancer have a 90% 5-year survival rate, while those with advanced or metastatic cancer have a 5-year survival rate of less than 5% (5), highlighting the need for innovative, long-lasting, and effective treatment options.

Immunotherapy is a relatively new anti-tumor treatment that stimulates the immune system to fight tumor cells by removing the immunosuppression of the immunological microenvironment. For B7 expressed on T-cells to bind to major histocompatibility complex 1 (MHC-1) molecules on antigen-presenting cells and activate T-cells to trigger an immunological response, the immune system must maintain a dynamic balance between activation and repression (6). However, T-cell activity may be suppressed when programmed death receptor-1 (PD-1), expressed on immune cells such as T-cells, binds to programmed death receptor-1 ligand (PD-L1), an inhibitory pathway molecule expressed on the surface of antigen-presenting cells (APC) or tumor cells (7). Tumor cells with increased PD-L1 expression have a reduced immunological microenvironment, impairing T-cell activity and preventing tumor cell eradication (8). PD-1/PD-L1 inhibitors work by obstructing the PD-1/PD-L1 pathway, killing tumor cells, and reversing immunosuppression to stimulate the immune system. Research into potential PD-1 and PD-L1 gene mutations in cervical cancer is necessary. (8).

The PD-1/PD-L1 axis is a well-known immune checkpoint pathway that allows cancer cells to evade the immune system, suppressing the immune response in various solid tumor types, including cervical cancer (9). Meng et al. (10) found that 60.82% (59/97) of patients had PD-1 expression in the tumor stroma of cervical cancer, while another study found that 46.97% (31/66) of patients had PD-1 expression (11). PD-L1 expression was infrequently detected in histologically normal cervical tissues but was found to be expressed in 34.4-59.6% of cervical cancer tissues (12). Reddy et al. (13) reported that 32 out of 93 (34.4%) cervical carcinoma samples had positive PD-L1 expression, with 28 out of 74 (37.8%) being squamous cell carcinomas of the cervix (SCCs), 2 out of 7

(28.6%) being adenosquamous carcinomas, and 2 out of 12 (16.7%) being endocervical adenocarcinomas. While many studies have investigated the expression of PD-L1 and PD-1 in cervical cancer, few have explored mutations in the PD-L1 and PD-1 genes in cervical cancers (13). The purpose of this study is to identify potential genetic mutations in the PD-L1 and PD-1 genes that may be associated with squamous cell carcinoma of the cervix. These genes play a crucial role in the immune system's ability to recognize and fight cancer cells. By identifying mutations in these genes, the study aims to gain a better understanding of the molecular mechanisms underlying this type of cancer, which may ultimately inform the development of new diagnostic and therapeutic strategies.

Methods and materials

Study design

This research employs a retrospective survey design to investigate the mutation of PD-L1 and PD-1 genes in subjects with cervical cancer.

Sample collection

A total of 20 cervical cancer tissue blocks were obtained from the pathology archive of Obafemi Awolowo University Teaching Hospital Complex (OAUTHC) in Ibadan, Oyo State, Nigeria. The subjects were aged 20-50 years and were of different religions, educational backgrounds, occupations, etc. All analyses were conducted in the Molecular Laboratory, at the University of Ibadan, in Oyo State, Nigeria. Frozen samples are stored at -20°C or -80°C to preserve nucleic acids, proteins, and other biomolecules. All twenty tissue blocks retrieved were formalin-fixed, paraffin-embedded tissue blocks consisting of ten benign cases and ten malignant tissues of the cervix

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Sample analysis:

DNA extraction

DNA extraction was performed using a modified Dellaporta DNA extraction protocol. Briefly, samples on slides were carefully scraped into a sterile mortar containing 500ml Dellaporta extraction buffer (100 mM Tris pH 8, 51 ml EDTA pH 8, 500 mM NaCl, 10mM mercaptoethanol) and ground with a sterile pestle. The mixture was collected in a sterile Eppendorf tube and 33 µl of 20% SDS was added, followed by brief vortexing and incubation at 65^{o}C for 10 minutes. At room temperature, 160 µl of 5 M potassium acetate was added, vortexed, and then 600 µl of Phenol Chloroform Isoamyl Alcohol in a 25:24:1 ratio was added. The mixture was vortexed and centrifuged at 10,000 g for 10 minutes. The supernatant was collected in another Eppendorf tube and 400 µl of cold isopropanol was added, gently mixed, and kept at 20^{o}C for 60 minutes. Centrifugation at 13000g for 10 minutes was done to precipitate the DNA after which the supernatant was gently decanted ensuring the pellet was not disturbed. The DNA was then washed with 500 µl of 70 % ethanol by centrifuging at 10000g for 10 minutes. The ethanol was decanted and the DNA was air-dried at room temperature until no trace of ethanol was visible in the tube. The pellet was then resuspended in 50 µl of Tris EDTA buffer to preserve and suspend the DNA (14).

Polymerase chain reaction

The PCR sequencing preparation cocktail for all PCR reactions consisted of 10 µl of 5x GoTaq colorless reaction, 3 µl of 25mM $MgCl_2$, 1 µl of 10 mM dNTPs mix, 1 µl of 10 pmol each primer (Table 1) and 0.3units of Taq DNA polymerase (Promega, USA) made up to 35 µl with sterile distilled water 15µl of DNA template was added. PCR was conducted in a GeneAmp 9700 PCR System Therma Cycler (Applied Biosystem Inc., USA) with a specific PCR profile for each primer as presented in the table 1.

Table 1: PCR profile and primers for PD1/PDL1

Gene Name	Primer Name	Primer Sequence	PCR profile
PDL1	PDL1F	CAGTCACCTCTGAACATGAA	An initial denaturation at 94^{o}C for 5 mins; followed by a 30 cycle consisting of 94^{o}C for 30s, 48^{o}C for 30s and 72^{o}C for 1 minute and a final termination at 72^{o}c for 10 minutes
	PDL1R	TTCCTCAGGATCTAATCTCCTA	
PDCD1	PDCD1F	CAGACAGGCCCTGGAAC	An initial denaturation at 94^{o}C for 5 mins; followed by a 30 cycle consisting of 94^{o}C for 30s, 48^{o}C for 30s and 72^{o}C for 1 minute and a final termination at 72^{o}c for 10 minutes
	PDCD1R	CGCAGGCTCTCTTTGATCT	

Integrity

The integrity of the amplified gene fragment was checked on a 1.5% agarose gel run to confirm amplification. The buffer (1XTAE buffer) was prepared and subsequently used to prepare a 1.5% agarose gel. The suspension was boiled in a microwave for 5 minutes. The molten agarose was allowed to cool to 60°C and stained with 3µl of 0.5 g/ml ethidium bromide (which absorbs invisible UV light and transmits the energy as visible orange light). A comb was inserted into the slots of the casting tray and the molten agarose was poured into the tray. The gel was allowed to solidify for 20 minutes to form the wells. The 1XTAE buffer was poured into the gel tank to barely submerge the gel. Two microliters (2 µl) of 10X blue gel loading dye (which gives color and density to the samples to make it easy to load into the wells and monitor the progress of the gel) was added to 4µl of each PCR product and loaded into the wells after the 100bp DNA ladder was loaded into well 1. The gel was electrophoresed at 120V for 45 minutes visualized by ultraviolet transillumination and photographed. The sizes of the PCR products were estimated by comparison with the mobility of a 100bp molecular weight ladder that was run alongside experimental samples in the gel (15).

Purification of amplified product

After gel integrity was confirmed, the amplified fragments were purified using ethanol to remove the PC reagents. Briefly, 7.6 µl of Na acetate 3M and 240 µl of 95% ethanol were added to each, about 40µl PC amplified product in a new sterile 1.5µl Eppendorf tube, mixed thoroughly by vortexing and kept at 20^{o}c for at least 30 minutes. Centrifugation for 10 minutes at 1300g and 4^{o}C followed by removal of supernatant (invert tube once) after which the pellet was washed by adding 150µl of 70% ethanol and mixed then centrifuged for 15 minutes at 7500g and 4^{o}c. Again, remove all supernatant (Invert tube on trash) invert the tube on paper tissue, and let it dry in the fume hood at room temperature for 10-15min then resuspend with 20µl of sterile distilled water and keep in 20^{o}c before sequencing. The purified fragments were checked on a 1.5% agarose gel run at a voltage of 110V for about 1 hour as previously to confirm the presence of the product and quantified using a Nano-Drop of model 2000 from Thermo Scientific (15).

Sequencing

The amplified fragments were sequenced using a genetic analyzer 3130xl sequencer from Applied Biosystem according to the manufacturer's

instructions, while the sequencing kit used was that of the Big Dye terminator v3.1 cycle sequencing kit Bio-Edit software and MEGA 6 was used for all genetic analysis.

Data analysis

Kapelan Bio-Imaging Solutions software version 2.7.2 was used to measure the length of the PD-L1 and PD-1 isoforms. Data obtained from the study were analyzed in Microsoft Excel using simple frequency and percentage. Results were presented using tables, charts, and graphs respectively.

Results

The clinical information and demographic characteristics of the participants under study are shown in Table 2. Twenty female participants with cervical cancer were enrolled in the study; 15% were in the 20–30 age range, 35% were in the 31–40 age range, and 50% were in the 41–50 age range. Most of the participants were married (75%), Christian (75%), had completed secondary school (45%), worked for the government (45%), had no family history of cancer (70%), had one or two children (55%), and resided in an urban region (75%).

Figure 1 is an agarose gel electrophorogram showing positive amplification of the PD-L1 gene, with a band size of approximately 200bp indicating positive amplification.

Plate 1 showed an H&E-stained micrograph of a benign cancer of the cervix, with the nucleus stained purple and cytoplasm-stained pink. The cervix exhibits rounded to oval structures with layers of stratified squamous epithelial cells at x100 magnification. At x400 magnification, the epithelial cells displayed distinct nuclei and no signs of dysplasia. Plate 2 is H&E-stained malignant cervical cancer tissue showing dysplasia, nuclear polymorphism, increased nucleo-cytoplasmic ratio, and disorganized tissue architecture.

Table 2: Demographic characteristics and clinical details of the subject

Variable	Frequency (%)
Age (years)	
20 – 30	3 (15.0%)
31 – 40	7 (35.0%)
41 – 50	10 (50.0%)
Marital status	
Single	2 (10.0%)
Married	15 (75.0%)
Divorced/Separated	3 (15.0%)
Educational status	
Primary	5 (25.0%)
Secondary	6 (30.0%)
Tertiary	9 (45.0%)
Occupation	
Government employed	9 (45.0%)
Self-employed	8 (40.0%)
Unemployed	3 (15.0%)
Religion	
Christian	15 (75.0%)
Muslim	4 (20.0%)
African traditional religion	1 (5.0%)
Residence	
Urban	15 (75.0%)
Rural	5 (25.0%)
Parity	
Nulliparous	6 (30.0%)
1 – 2 births	11 (55.0%)
3 & above	3 (15.0%)
Nature of cancer	
Malignant	10 (50.0%)
Benign	10 (50.0%)

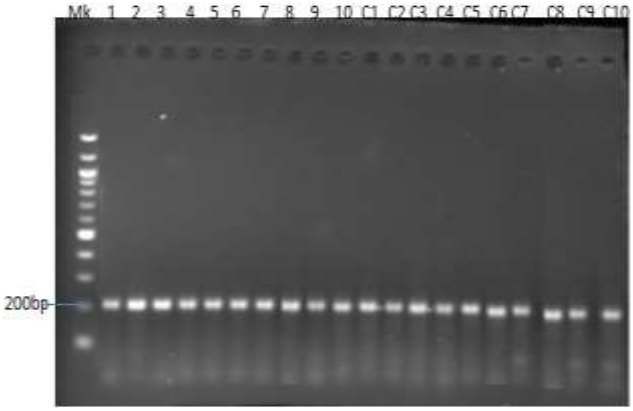

Figure 1: Agarose gel electrophorogram showing positive amplification of PD-L1 gene

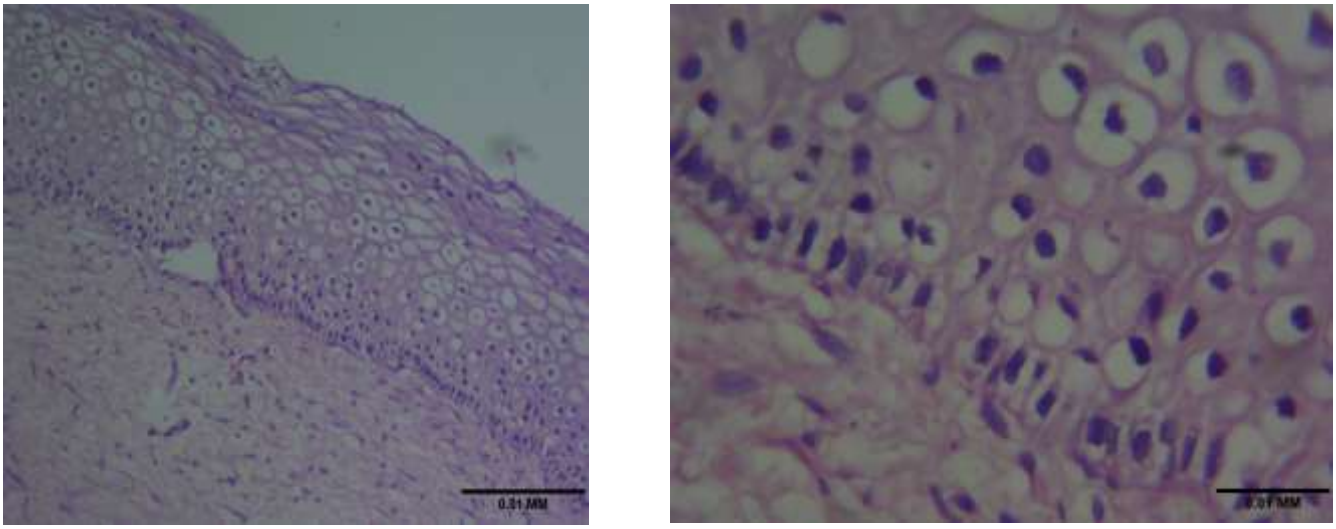

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Plate 1: H&E-stained micrograph of a benign cancer of the cervix

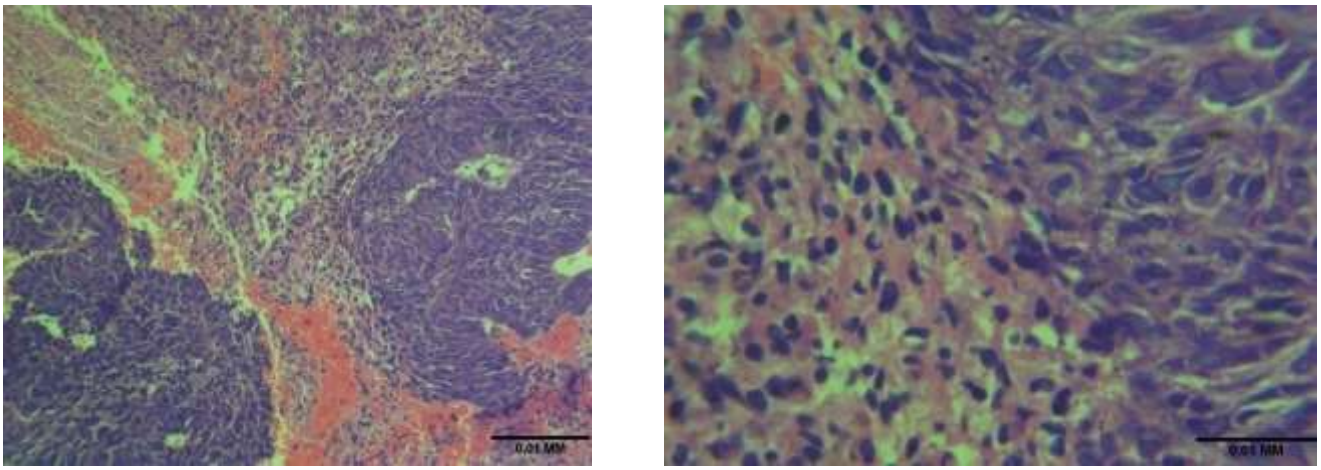

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Plate 2: H&E-stained photomicrograph of malignant cervical cancer tissue

Table 3 presents the mutation type, locations along the nucleotide sequence, and frequency along the PD-1 and PD-L1 gene regions. The results for PD-L1 showed mutations of G to C in location 18 (17:3) changing ATG (methionine) to ATC (isoleucine), mutation of G to C in location 126 (17:3) changing glycine to alanine, and transition mutation of A to G in location 202(10:10) changing aspartate to asparagine. On the other hand, PD1 displayed mutations from C to T in location 31(16:3) changing proline to serine, and mutation from C to T in location 161(11:8) retaining alanine. Figure 2 displays an agarose gel electrophoresis image demonstrating positive amplification of the PD1 gene, with a band size of approximately 300bp indicating positive amplification. Figure 3 illustrates the percentage of single nucleotide polymorphism along the PD-L1 and PD1 gene regions in cervical cancer. The results show that only a transition mutation (100%) was present in the PD1 gene, while 37.5% transversion and 62.5% transition mutations were present in the PD-L1 gene. Indel mutations were absent in both genes.

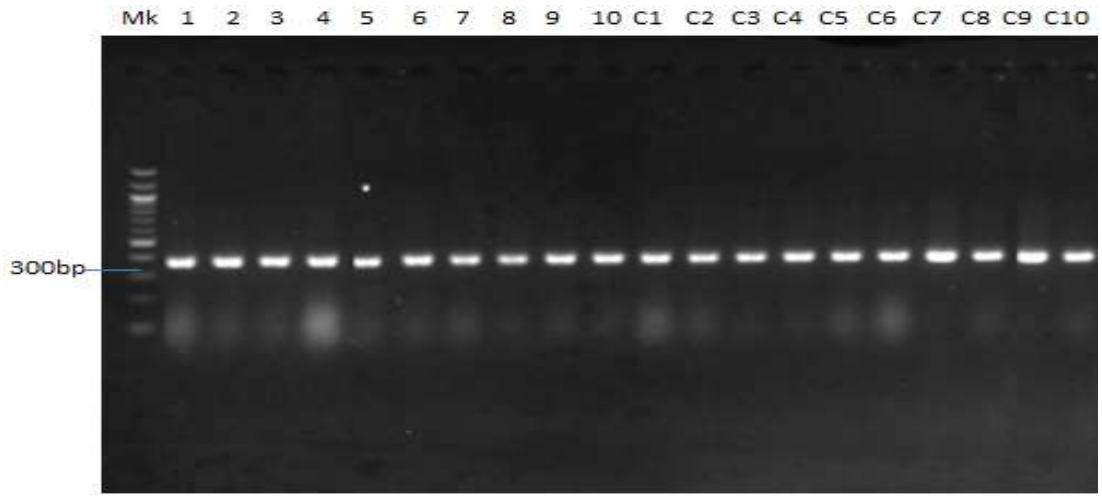

Figure 2: Agarose gel electrophorogram showing positive amplification of PD1 gene

Table 3: Mutation types in the PD-L1 and PD-1 genes in cervical cancer

Gene Type	Mutation Type	Description	Location/ Frequency	Mutation Type Description
PD-L1	Transversion	G: C	18(17:3)	Missense mutation changing methionine to isoleucine
		G: C	126(17:3)	Missense mutation changing glycine to alanine
	Transition	A: G	202(10:10)	Missense mutation changing aspartate to asparagine
PD1	Transversion			
	Transition	C: T	31(16:3)	Missense mutation changing proline to serine.
		C: T	161(11:8)	Silent mutation retaining alanine

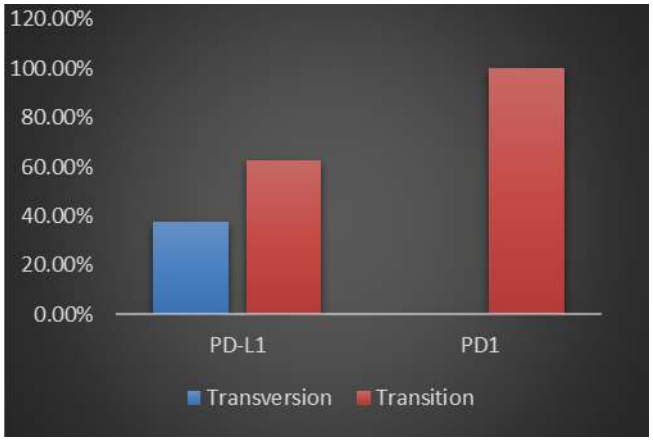

Figure 3: Percentage of single nucleotide polymorphism along the PD-L1 and PD1 gene regions in cervical cancer.

Figure 4 illustrates the percentage of functional mutations along the PD-L1 and PD-1 genes, respectively. The results reveal that PD-L1 displays 100% missense mutation, while PD-1 showed 20% missense mutation and 80% silent mutation. Nonsense mutations were absent in both the PD-L1 and PD1 genes.

Discussion

Advances in our understanding of the underlying mechanisms of gene mutation in cervical cancer, the development of targeted therapies (gene therapies), the improvement of early detection methods, and the creation of personalized treatment plans for those affected by the disease depend on our ability to identify the type, frequency, and location of mutations in the PD-1/PD-L1 genes.

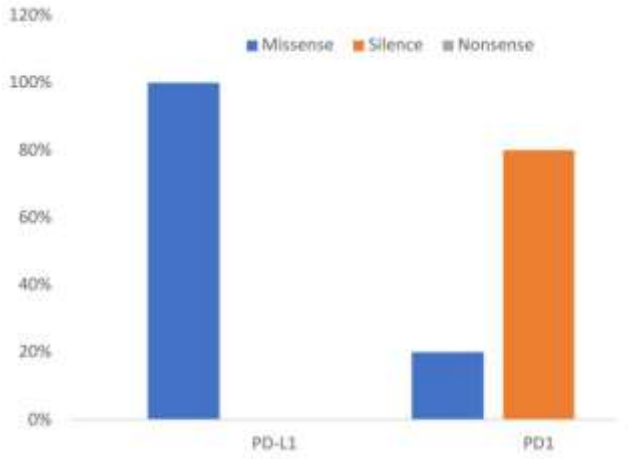

Figure 3: Percentage of functional mutations along the PD-L1 and PD-1 genes in cervical cancer.

In this study, mutations were found in the PD-L1 and PD-1 genes in cases of cervical cancer. PD-L1 is expressed on the surface of tumor cells, while PD-1 is expressed on the surface of immune cells. Mutations in the PD-L1 and PD-1 genes in cervical cancer may be due to the overexpression of the PD-L1 gene on PD1, leading to immune evasion.

Human Papillomavirus (HPV) is the primary cause of cervical cancer. Variations in PD-L1 and PD1 interactions with the immune system may be caused by HPV's ability to manipulate the immune response through the upregulation of PD-L1 on infected cells (16). This finding is consistent with a previous study suggesting that viruses can evade the immune response by overexpressing PD-L1 (17). According to Alsaab et al. (18), there is a correlation between a higher risk of cervical cancer and the interaction between PD-L1 and PD-1 on their signal pathways. A plausible theory is that mutations in the PD-L1 and PD-1 signal pathways lower the immune response, accelerating tumor heterogeneity and increasing the severity of cervical cancer.

In the human genome, single nucleotide polymorphisms (SNPs) are the most common genetic variants, with a minor allele frequency (MAF) of more than 1% occurring approximately once every 300 base pairs on average (19). According to this study, the most common SNPs in the PD-L1 and PD-1 genes were transition mutations. In a study on cervical squamous cell carcinoma (SCC), 80% (56/70) of patients had PD-L1 gene expression (20). In the TCGA database for cervical SCCs, the amplification or gain of PD-L1 and PDCD-1 were found in 28 of 129 (22%) cases (21). These findings indicate the widespread expression of both PD-L1 and PD-1 in the stroma and tumor cells of cervical cancer, suggesting both pathways as viable targets for PD-1/PD-L1 inhibitor therapy. The results of this investigation are consistent with earlier research that found transversion mutations (39.9%) to be the least common mutation in the PD-L1 gene and transition mutations (60.1%) to be the most common SNPs mutation (19,22). This suggests that the SNPs in the PD-L1 gene exhibit a transition mutation pattern, possibly because transversion mutations have a greater impact on transcription factor binding due to changes in the minor groove width and roll of DNA (23).

The results of this study revealed that missense mutations converting methionine to isoleucine, glycine to alanine, and aspartate to asparagine were the most common functional mutations in the PD-L1 gene. In contrast, silent mutations were predominant in the PD-1 gene, specifically retaining alanine, with a missense mutation altering proline to serine following closely. This finding is in line with Zhang et al. (24), who also observed silent and missense mutations in the PD-1 gene.

Missense mutations being the most prevalent in the PD-L1 gene modify the protein's amino acid sequence and enhance binding to PD-1. This could potentially enhance immune evasion by cancer cells leading to increased tumor growth and progression (24). This is consistent with previous research suggesting that missense mutations can impact PD-L1 gene function, affecting programmed cell death control and protein synthesis (17,25-26). The specific amino

acid substitution resulting from a missense mutation can influence protein structure and function, potentially leading to abnormal cellular processes and contributing to the development or progression of squamous cell carcinoma of the cervix (9). On the other hand, silent mutations do not alter the protein sequence, but may still affect function through various pathways like RNA splicing, translation efficiency, or stability (27).

In this study, transition mutation was the most prominent, occurring in 100% of cases on the PDCD-1 gene. This may be attributed to the different conformations of purines and pyrimidines, as purines have a bicyclic structure while pyrimidines have a single-ring structure (13). Consequently, the process of transversion is likely more complex than that of transition. This finding is consistent with previous research that identified transition mutations as the primary mutation in the PD-1 gene (13,29).

The observation of PD-L1 protein expression in 73% of cervical cancer tissues, with high expression in 44% of cases, was supported by histological findings from Heeren et al. (29). This further validates the evidence obtained in the mutational pattern of PD-L1 and PD-1 in cervical cancer. Another study found a significant correlation between PD-L1 expression and both tumor size and lymph node metastasis. Additionally, immunohistochemistry (IHC) staining revealed a notable association between PD-L1 expression and mutations in the PD-L1 gene.

The PD-L1 and PD-1 axis was activated in 56% of cases, indicating an immune suppressive microenvironment (30). These results suggest that the PD-L1/PD1 axis plays a crucial role in immune evasion and the progression of cervical cancer. Mutational analysis can help identify patients who may benefit from immunotherapy

Conclusion

In conclusion, mutations were found in both the PD-L1 and PD-1 genes in cervical cancer cells. There were more mutations in the PD-L1 gene compared to the PD-1 gene, suggesting potential therapeutic targets for PD-1/PD-L1 inhibitors. As PD-1/PD-L1 blockade therapy is expected to become a prominent cancer immunotherapy technique soon, targeting PD-1 or PD-L1 in cancer immunotherapy may effectively induce long-lasting anticancer immune responses with reduced toxicity in various cancers, particularly cervical cancer. Further studies utilizing a larger sample size are required to confirm the findings of this study.

Abbreviations

DNA: Deoxyribonucleic acid
H&E: Hematoxylin and Eosin
HPV: Human papilloma virus
IHC: Immunohistochemistry
MAF: Minor allele frequency
PD-L1 :Programmed cell death ligand 1
PD1: Programmed death-1
SNPs: Single nucleotide polymorphisms
SCC: Squamous cell carcinoma

Contributions

V.O.E conceptualized the manuscript. The methodology was developed by E.O.U., M.A.H., and A.O.A. Formal analyses and visualization were conducted by E.A.O, M.O.D, M.A.H. and A.O.A. Resources were contributed by all authors. E.A.O and E.O.U. wrote the original draft, while review and editing were done by all authors. Supervision was provided by V.O.E. E.A.O. was responsible for coordination with the co-authors and submission of the article. All authors have read and approved the final manuscript.

Ethical considerations

Ethical approval for this study was obtained from the Health Research Ethics Committee, College of Medicine and Health Sciences, Afe Babalola University, Ado-Ekiti, Ekiti State, Nigeria. Informed consent was obtained from

each participant who participated in the study. All experiment in this study was performed following the Declaration of Helsinki.

Data availability statement

All the data used for the analysis is available in the paper.

Conflicts of interest

The authors declare no financial or non-financial competing interests.

Funding statement

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References

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. Cancer Journal Clinic, 2021; 68(1):394–424.
2. Ekundina VO, Ayowole OF, Ejelue CC, Oladele AA, Sanya O, Eze GI. Assessment of P53, BCL-2 and CD34 in premalignant and malignant cervical lesions. J. Krishna Institute Med. Sci. Uni. 2022; 11(2): 31-45.
3. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2020. CA: Cancer Journal Clinics, 2020; 70(2): 17–30.
4. Wright JD, Matsuo K, Huang Y, Tergas AI, Hou JY, Khoury-Collado F. Prognostic performance of the 2018 international federation of gynecology and obstetrics cervical cancer staging guidelines. Obstetrics & Gynaecology, 2019; 134(9):49–57.
5. Huang W, Liu J, Xu K, Chen H, Bian C. PD-1/PD-L1 inhibitors for advanced or metastatic cervical cancer: From bench to bed. Frontiers in Oncology, 2020; 12(4):352-357.
6. Dibbern ME, Bullock TN, Jenkins TM, Duska LR, Stoler MH, Mills AM. Loss of MHC class I expression in HPV-associated cervical and vulvar neoplasia: A potential mechanism of resistance to checkpoint inhibition. American Journal of Surgery & Pathology, 2020; 44(2):1184–1191.
7. Chen Z, Pang N, Du R, Zhu Y, Fan L, Cai D. Elevated expression of programmed death-1 and programmed death ligand-1 negatively regulates immune response against cervical cancer cells. Mediators Inflammation, 2021; 16(1):482-495.
8. Burley M, Roberts S, Parish JL. Epigenetic regulation of human papillomavirus transcription in the productive virus life cycle. Seminars in Immunopathology, 2020; 42(1):159–171
9. Liu J, Chen Z, Li Y, Zhao W, Wu J, Zhang Z. PD-1/PD-L1 Checkpoint Inhibitors in Tumor Immunotherapy. Frontiers in Pharmacology, 2021; 12(3):731-736.
10. Meng Y, Liang H, Hu J, Liu S, Hao X, Wong MSK. PD-L1 Expression correlates with tumor infiltrating lymphocytes and response to neoadjuvant chemotherapy in cervical cancer. Journal of Cancer, 2019; 9(1):2938–2945.
11. Feng YC, Ji WL, Yue N, Huang YC, Ma XM. The relationship between the PD-1/PD-L1 pathway and DNA mismatch repair in cervical cancer and its clinical significance. Cancer Management Research, 2020; 10(1):105–113.
12. Enwere EK, Kornaga EN, Dean M, Koulis TA, Phan T, Kalantarian M. Expression of PD-L1 and presence of CD8-positive T cells in pre-treatment specimens of locally advanced cervical cancer. Modern Pathology, 2019; 30(1):577–586.
13. Reddy OL, Shintaku PI, Moatamed NA. Programmed death-ligand 1 (PD-L1) is expressed in a significant number of the uterine cervical carcinomas. Diagnostic Pathology, 2021; 12(2):45-51.
14. Ekundina VO, Ogazi M, Oladele AA, Sanya J. The Predictive Significance of

P16INK4A, Ki-67 and Ck7 Expressions in the Progression to SCC of the Cervix. International Journal of Science, Engineering and Technology, 2021; 8(1):195-208.
15. Ekundina VO, Omon EA. Genomic analysis of BAX and Bcl-2 gene mutations in human papilloma virus-associated squamous cell carcinoma of the cervix. Surg Exp Pathol. 2024; 7:11.
16. Snijders PJ, Steenbergen RD, Heideman DA, Meijer CJ. HPV-mediated cervical carcinogenesis: concepts and clinical implications. The Journal of Pathology, 2019; 208 (2):152–164.
17. Su Y, Sai Y, Zhou L, Liu Z, Du P, Wu J, Zhang J. Current insights into the regulation of programmed cell death by TP53 mutation in cancer. Frontiers in Oncology, 2020; 12(2):427-434.
18. Alsaab HO, Sau S, Alzhrani R, Tatiparti K, Bhise K, Kashaw SK, Iyer AK. PD-1 and PD-L1 Checkpoint Signaling Inhibition for Cancer Immunotherapy: Mechanism, Combinations, and Clinical Outcome. Frontiers in Pharmacology, 2021; 8(1):561-565.
19. Wagner M, Tupikowski K, Jasek M, Tomkiewicz A, Witkowicz A, Ptaszkowski K, Karpinski P, Zdrojowy R, Halon A, Karabon L. SNP-SNP Interaction in Genes Encoding PD-1/PD-L1 Axis as a Potential Risk Factor for Clear Cell Renal Cell Carcinoma. Cancers, 2020; 12(12):3521-3526.
20. Mezache L, Paniccia B, Nyinawabera A, Nuovo GJ. Enhanced expression of PD L1 in cervical intraepithelial neoplasia and cervical cancers. Modern Pathology, 2021; 28(3):1594-1602.
21. Dijkstra KK, Voabil P, Schumacher TN, Voest EE. Genomics and transcriptomics-based patient selection for cancer treatment with immune checkpoint inhibitors: a review. Journal of American Medical Association Oncology, 2022; 2(1), 1490–1495
22. Fischer A, Bankel L, Hiltbrunner S, Rechsteiner M, Rüschoff JH, Rushing EJ, Britschgi C, Curioni-Fontecedro A. Mutational Landscape and Expression of PD-L1 in Patients with Non-Small Cell Lung Cancer Harboring Genomic Alterations of the MET gene. Target Oncology, 2022; 17(6):683-694.
23. Robert F, Pelletier J. Exploring the Impact of Single-Nucleotide Polymorphisms on Translation. Front. Genet. 2018; 9:507.
24. Zhang Z, Miteva MA, Wang L, Alexov E. Analyzing effects of naturally occurring missense mutations. Computational Mathematic Methods in Medicine, 2022; 12(2):827-834.
25. Zhang T, Liu H, Jiao L, Zhang Z, He J, Li L, Qiu L, Qian Z, Zhou S, Gong W, Meng B, Ren X, Zhang H, Wang X. Genetic characteristics involving the PD-1/PD-L1/L2 and CD73/A2aR axes and the immunosuppressive microenvironment in DLBCL. J Immunother Cancer. 2022; 10(4):e004114.
26. Shen X, Zhang L, Li J, Li Y, Wang Y and Xu ZX. Recent Findings in the Regulation of Programmed Death Ligand 1 Expression. Front. Immunol. 2019; 10:1337.
27. Patel UR, Gautam S, Chatterji D. Unraveling the Role of Silent Mutation in the ω-Subunit of Escherichia coli RNA Polymerase: Structure Transition Inhibits Transcription. ACS Omega, 2019; 4(18):17714-17725.
28. Thomas BT, Ogunkanmi LA, Iwalokun BA, Popoola OD. Transition-transversion mutations in the polyketide synthase gene of Aspergillus section Nigri. Heliyon, 2019; 5(6):1881-1888.
29. Heeren AM, Punt S, Bleeker MC, Gaarenstroom KN, Velden J. Prognostic effect of different PD-L1 expression patterns in squamous cell carcinoma and adenocarcinoma of the cervix. Modern Pathology, 2021; 29(3):753-763.
30. Yang W, Lu YP, Yang YZ, Kang JR, Jin YD. Expressions of programmed death (PD)-1 and PD-1 ligand (PD-L1) in

cervical intraepithelial neoplasia and cervical squamous cell carcinomas are of prognostic value and associated with human papillomavirus status. Journal of Obstetrics & Gynaecology Research, 2019; 43(3):1602-1612.