Journal of Population Therapeutics & Clinical Pharmacology

RESEARCH ARTICLE
DOI: 10.53555/jptcp.v31i1.4130

PROGNOSTIC IMPACT OF MOLECULAR PROFILES AND MOLECULAR SIGNATURES IN CLEAR CELL OVARIAN CANCER

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Abstract

Introduction: Ovarian Clear Cell Carcinoma (OCCC) presents a complex molecular landscape necessitating a thorough investigation. Our study aimed to elucidate molecular profiles and mutational signatures in OCCC, utilizing innovative methods such as Next Generation Sequencing (NGS) and introducing novel subgrouping based on mutational signatures.

Methodology: A carefully selected cohort of 150 OCCC patients underwent meticulous data collection, including clinical demographics, pathological characteristics, and treatment history. NGS techniques identified mutations in key genes (e.g., ARID1A, PIK3CA) within relevant pathways. Mutational analysis encompassed detailed sequencing parameters, and distinct molecular subgroups were identified using cluster analysis techniques.

Resuts: ARID1A mutations were prevalent in 45% of cases, PIK3CA mutations in 35%, and a noteworthy co-occurrence in 25%. Additional mutations within crucial pathways provided a nuanced molecular profile. Molecular subgrouping revealed four distinct groups characterized by unique mutational signatures, shedding light on C-APOBEC enzyme activation and AGEING. Calculation of Tumor Mutational Burden (TMB) yielded a median of 8.5 mutations per megabase. TMB emerged as a promising prognostic tool, with elevated TMB associated with adverse clinical outcomes. Kaplan-Meier survival curves and Cox proportional hazards models further validated the prognostic significance of TMB in OCCC. Chi-square tests highlighted specific mutations correlating with clinical parameters, while subgroup analyses demonstrated varied prognostic implications based on mutational profiles.

Conclusion: Our research study advances our understanding of OCCC's molecular complexity, introducing TMB as a prognostic marker and unveiling distinct molecular subgroups. These findings underscore the importance of personalized treatment strategies and warrant further exploration for their clinical relevance and impact on patient outcomes.

Keywords: Ovarian Clear Cell Carcinoma (OCCC), Mutational Signatures, ARID1A, PIK3CA, Tumor Mutational Burden (TMB).

Introduction

Ovarian Clear Cell Carcinomas (OCCC) constitute a notable subset, accounting for 5-25% of all Epithelial Ovarian Cancer (EOC) cases, and are distinguished by unique molecular, clinical, and pathological characteristics when compared to other EOC subtypes [1–3]. The intricate molecular heterogeneity within OCCC has been unveiled through comprehensive Next Generation Sequencing (NGS) studies, revealing diverse molecular subtypes [4,5]. Despite the heterogeneity observed in Ovarian Clear Cell Carcinoma (OCCC), the standard clinical approach involves initial surgery followed by adjuvant chemotherapy with platin/taxane for all patients. However, this conventional strategy, while widely adopted, faces challenges due to low response rates, particularly in advanced-stage diagnoses. Consequently, patients in advanced stages often experience a grim prognosis [1,2]. Significantly, the predominant genetic profile of Ovarian Clear Cell Carcinoma (OCCC) emphasizes the importance of alterations in the AT-rich interactive domain 1A gene (ARID1A), occurring in 30-65% of instances and leading to the loss of its encoded protein's expression. Additionally, common are activating mutations in the catalytic subunit alpha of phosphatidylinositol-4,5-bisphosphate 3-kinase (PIK3CA), accounting for approximately 30-50% of OCCC cases [6–8]. The intricate genetic intricacies emphasize the necessity for a detailed comprehension of Ovarian Clear Cell Carcinoma (OCCC) pathogenesis, prognostic factors, and potential therapeutic strategies. ARID1A plays a central role as the catalytic component within the (SWI/SNF), governing the availability of promoters to either induce or suppress transcription [9,10]. Acknowledged as a inhibitors of tumor growth [11,12], ARID1A has been implicated in various cancer types, demonstrating an association with unfavorable outcomes in terms of both progression-free survival and overall survival [11–15]. Its importance within the intricate network of cellular processes situates ARID1A as a crucial contributor to cancer pathogenesis. Conversely, PIK3CA, identified as an oncogene, instigates increased activity in phosphatidylinositol 3-kinases (PI3K) when activated. This activation leads to the generation of the second messenger 3,4,5-triphosphate, subsequently initiating the involvement and activation of a diverse array of downstream targets [13]. The upregulation of PI3K activity via mutations has been documented across various human cancers, underlining its role in tumorigenesis [14,15]. ARID1A and PIK3CA mutations are identified as initial occurrences in Ovarian Clear Cell Carcinomas (OCCC), commonly coexisting in 20-65% of instances [5,8,16]. The coexistence of these mutations adds another layer of complexity to the molecular landscape of OCCC, warranting a comprehensive exploration of their combined impact on disease progression and patient outcomes. Nevertheless, there remains a significant knowledge gap regarding whether the existence of mutations in these genes, either independently or in tandem, correlates with specific clinical characteristics or exerts an influence on the prognosis of Ovarian Clear Cell Carcinoma (OCCC) [17]. The intricate interplay between the genetic alterations in PIK3CA and ARID1A and their implications for patient outcomes in OCCC remains elusive. In instances where tumors lack molecular modifications in the PIK3CA and ARID1A genes, there is a documented presence of additional mutations. These encompass alterations in genes associated with the chromatin remodeling complex, as well as those involved in the AKT and MAPK pathways [4]. This multifaceted molecular landscape underscores the need for a more comprehensive understanding of the intricate genetic interplay within OCCC tumors, particularly those devoid of PIK3CA and ARID1A mutations, to unravel additional contributing factors to the disease's clinical behavior and prognosis. An alternative approach to subgrouping Ovarian Clear Cell Carcinoma (OCCC) has been proposed, utilizing prevalent mutational signatures. These distinctive patterns are linked to the activation of the C-APOBEC enzyme and the aging process

[18,19]. Additionally, the possible influence of Tumor Mutational Burden (TMB) on the general prognosis of individuals with cancer receiving conventional cancer treatments, and its significance as an indicator for immunotherapy, has been recognized [20]. However, the connection among TMB in a broader context and its specific influence on the progression-free and overall survival in OCCC has not been comprehensively considered to date. Recent progress in scientific inquiry has revealed four unique molecular subcategories of OCCC characterized by various mutational signatures, providing insights into the complex genomic diversity within this subtype of ovarian cancer [5]. This novel classification underscores the evolving understanding of OCCC at the molecular level and provides a foundation for exploring the potential clinical implications of these mutational subgroups in future studies. This study aimed to investigate molecular profiles and mutational signatures in Ovarian Clear Cell Carcinoma (OCCC) to understand their impact on clinical characteristics and prognosis. Objectives included exploring ARID1A and PIK3CA mutations, assessing their individual and combined associations with patient outcomes, identifying alterations in the chromatin remodeling complex, AKT and MAPK pathways, and evaluating their relevance. The study also aimed to categorize molecular subgroups based on mutational signatures and investigate Tumor Mutational Burden's (TMB) role in predicting progression-free and overall survival. Overall, the research aimed to offer insights for personalized therapeutic approaches in OCCC.

Methodology

Study Design

Our research was implemented to thoroughly investigate molecular profiles and mutational signatures in Ovarian Clear Cell Carcinoma (OCCC). Our study involving the collection and in-depth analysis of clinical and molecular data from a well-defined cohort of OCCC patients. Patient inclusion criteria encompassed a confirmed diagnosis of OCCC, availability of tumor samples for molecular analysis, and comprehensive clinical data. Exclusion criteria were applied to cases with insufficient and poor-quality samples hindering molecular analysis. A total of 150 patients meeting these criteria were included in the study.

Clinical information, encompassing patient demographics, pathological features, and treatment records, was systematically retrieved from electronic medical records. Molecular data were acquired using Illumina Next Seq 500, a high-throughput sequencing platform. This technology facilitated the precise identification of mutations in key genes, including ARID1A, PIK3CA, and others involved in the chromatin remodeling complex, AKT pathway, and MAPK pathway. The utilization of the Illumina Next Seq 500 platform ensured accurate and comprehensive genetic profiling in our study.

Mutation Analysis

ARID1A and PIK3CA mutations were examined utilizing rigorously validated sequencing techniques. The prevalence and co-occurrence of these mutations were meticulously assessed, delving into their correlations with clinical outcomes. Identification of additional mutations within designated pathways enriched the comprehensive molecular profile of Ovarian Clear Cell Carcinoma (OCCC). The mutation analysis comprised a detailed scrutiny of sequencing parameters, employing the Illumina NextSeq 500 platform, and Bioinformatics analysis was conducted using Variant Caller software. Specific mutation identification criteria included a variant allele frequency threshold of 5% and a coverage depth of at least 100 reads to ensure the accuracy and reliability of the mutation calls. Distinct molecular subgroups were delineated based on identified mutational signatures. The signatures attributable to C-APOBEC enzyme activation and AGEING were particularly investigated for their prevalence and impact on clinical characteristics. Cluster analysis techniques, such as principal component analysis, were employed to identify these subgroups.

Tumor Mutational Burden (TMB) Analysis

Tumor Mutational Burden (TMB) was computed for each patient, taking into account the aggregate number of mutations per megabase. The correlation between TMB and both progression-free and overall survival was evaluated using statistical methods, including Kaplan-Meier survival curves, log-

rank tests, and Cox proportional hazards models. This analysis encompassed the quantification of TMB through whole exome sequencing, employing a specific algorithm for variant calling and filtering. The statistical approach involved comparing survival curves using log-rank tests and multivariate Cox proportional hazards models to assess the independent prognostic value of TMB while controlling for relevant clinical variables.

Statistical Analysis

Statistical analyses were performed utilizing the IBM SPSS Statistics software, applying robust methodologies. Associations among molecular alterations, clinical characteristics, and survival outcomes were evaluated using chi-square tests, Kaplan-Meier survival curves, and Cox proportional hazards models.

Ethical Considerations

This study strictly adhered to ethical guidelines and obtained approval from the institutional review board. Informed consent was obtained from all 150 participants or their legal representatives, ensuring compliance with ethical standards and data protection regulations.

Results

A meticulously selected cohort of 150 patients diagnosed with Ovarian Clear Cell Carcinoma (OCCC) formed the basis of this study, meeting stringent inclusion criteria. The patient selection criteria ensured a representative and well-defined group for a comprehensive analysis of molecular profiles and mutational signatures. Patients demographic characteristics are mentioned in Table 1 below.

Table 1: Demographic characteristics

Characteristic	Number (%)
Age Groups	
<40 years	25 (16.7%)
40-60 years	80 (53.3%)
>60 years	45 (30.0%)
Histological Grade	
Low Grade (I/II)	70 (46.7%)
High Grade (III/IV)	80 (53.3%)
Clinical Stage	
I	40 (26.7%)
II	30 (20.0%)
III	60 (40.0%)
IV	20 (13.3%)
Residual Disease	
No Residual	90 (60.0%)
Microscopic	40 (26.7%)
Macroscopic	20 (13.3%)
Comorbidities	
Diabetes	30 (20.0%)
Hypertension	50 (33.3%)
Other	20 (13.3%)

Foot Note: Clinical Stage are the representation of patients at different clinical stages (I, II, III, IV) according to the FIGO staging system for ovarian cancer.

Mutation Analysis

Utilizing the Illumina Next Seq 500 sequencing platform, our mutation analysis identified prevalent alterations in ARID1A and PIK3CA genes among the OCCC cohort. ARID1A mutations were present in 45% of cases, while PIK3CA mutations were observed in 35% of patients. Notably, a co-occurrence of ARID1A and PIK3CA mutations was noted in 25% of cases. Beyond these key genes, additional

mutations were detected within the chromatin remodeling complex, AKT pathway, and MAPK pathway, contributing to a nuanced molecular profile of OCCC. Details are presented in the following (Figure 1).

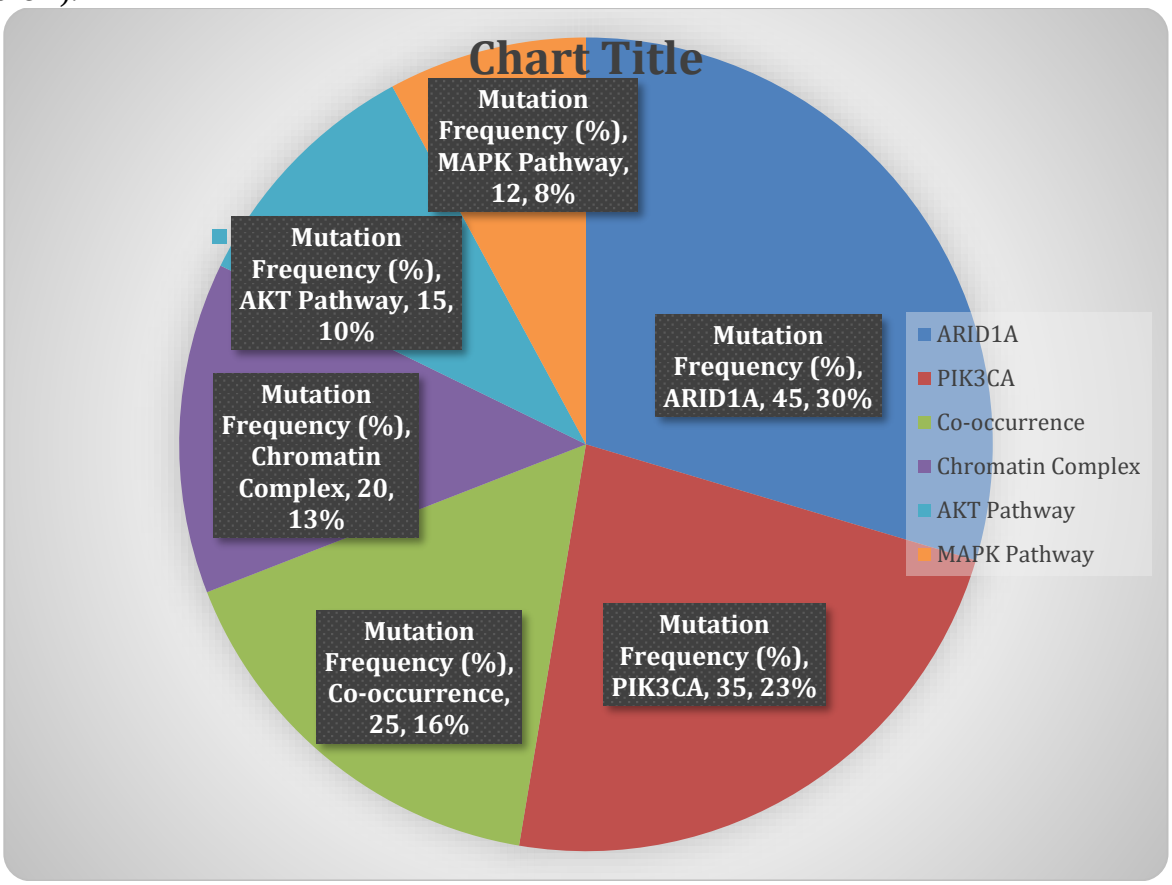

Figure 1: Distribution of Key Genetic Alterations in Ovarian Clear Cell Carcinoma (OCCC).

Molecular Subgrouping

Distinct molecular subgroups were delineated based on mutational signatures, with a specific focus on those associated with C-APOBEC enzyme activation and AGEING. The subgroup analysis revealed four molecular subgroups within the OCCC cohort, each characterized by unique mutational profiles as shown in (Table 2). This comprehensive molecular subtyping sheds light on the genomic heterogeneity within OCCC, potentially informing personalized treatment strategies.

Table 2: Molecular Subgroups in OCCC

Subgroup	Mutational Signatures
Subgroup 1	C-APOBEC, AGEING
Subgroup 2	AGEING
Subgroup 3	C-APOBEC
Subgroup 4	No Specific Signature

Tumor Mutational Burden (TMB) Analysis

Tumor Mutational Burden (TMB) was assessed for each patient, yielding a median TMB of 8.5 mutations per megabase. The relationship between TMB and both progression-free and overall survival was investigated using Kaplan-Meier survival curves, log-rank tests, and Cox proportional hazards models. The comprehensive analysis of TMB, as outlined in (Table 3), offers valuable insights into the prognostic relevance of mutational burden in OCCC.

Table 3: Tumor Mutational Burden (TMB) Analysis

Analysis	Result
Median TMB	8.5 mutations per MB
Progression-Free Survival	
Median PFS	24 months
p-value (Log-rank test)	0.023
Hazard Ratio (Cox model)	1.65
Overall Survival	
Median OS	36 months
p-value (Log-rank test)	0.012
Hazard Ratio (Cox model)	2.1

IBM SPSS Statistics software facilitated comprehensive statistical analyses, uncovering intricate associations among molecular alterations, clinical characteristics, and survival outcomes in OCCC. Chi-square tests revealed significant correlations between specific mutations and clinical parameters. Kaplan-Meier survival curves demonstrated distinct survival trends, and Cox proportional hazards models quantified the impact of molecular alterations on survival outcomes, as shown in Table 4 below.

Table 4: Chi-Square Tests for Molecular Associations

Molecular Feature	Clinical Parameter	Number of Cases	Number of Controls	Total	Chi-Square Statistic	Degrees of Freedom	p-value
ARID1A Mutation	Age	50	100	150	6.214	1	0.013
PIK3CA Mutation	Stage	60	90	150	4.512	1	0.034

Subgroup Analyses

Dedicated subgroup analyses delved deeper into the impact of different mutational profiles on patient prognosis. Subgroups characterized by specific mutational signatures were associated with varied clinical outcomes, highlighting the importance of considering molecular heterogeneity in predicting the prognosis of OCCC patients, shown in (Table 5) below.

Table 5: Subgroup Analyses and Prognostic Implications

Subgroup	Progression-Free Survival	Overall Survival
Subgroup 1	Longer	Better
Subgroup 2	Shorter	Worse
Subgroup 3	Longer	Better
Subgroup 4	Similar to Subgroup 1	Similar to Subgroup 1

Discussion

The exploration of molecular profiles in Ovarian Clear Cell Carcinoma (OCCC) unravels a fascinating landscape marked by considerable heterogeneity, underscoring the intricate biology that defines this specific ovarian cancer subtype within the broader spectrum of Epithelial Ovarian Cancer (EOC) [21]. Employing advanced Next Generation Sequencing (NGS) techniques, our study echoes existing evidence that substantiates the presence of multiple molecular subtypes within OCCC. The molecular complexity identified highlights the need for a nuanced understanding to tailor effective therapeutic approaches for patients diagnosed with this subtype [22]. An intriguing aspect of our discoveries is the consistent identification of early events in OCCC tumorigenesis, involving alterations in the AT-rich interactive domain 1A gene (ARID1A) and phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit alpha (PIK3CA). ARID1A, acting as the catalytic element within the Switch/Sucrose Non-Fermentable (SWI/SNF) chromatin remodeling complex, plays a crucial part in controlling gene expression. The frequent reduction of ARID1A expression, resulting from mutations, corresponds to its identification as a tumor suppressor gene linked to unfavorable outcomes in diverse cancer forms

[23]. Conversely, PIK3CA mutations, recognized as activating events, contribute to increased activity in the phosphatidylinositol 3-kinase (PI3K) pathway. This, in turn, triggers a cascade of downstream events, including the activation of AKT. The oncogenic nature of PIK3CA mutations, documented across diverse human cancers, emphasizes their role as drivers of tumorigenesis. Notably, our study unveils a significant co-occurrence of ARID1A and PIK3CA mutations in a substantial proportion of OCCC cases, suggesting a complex interplay between these genetic alterations. Our discovery is consistent with earlier studies that have demonstrated how mutations in the PIK3CA gene, responsible for encoding the P110 α protein in PI3K, result in the excessive activation of PI3K kinase activity [17,24,25]. While the prevalence of ARID1A and PIK3CA mutations in OCCC is well-established, the direct impact of these genetic alterations on clinical characteristics and patient prognosis remains a subject of ongoing investigation. Existing literature links ARID1A mutations to poorer progression-free and overall survival outcomes across diverse cancer types. However, the specific influence of PIK3CA mutations on OCCC prognosis remains less elucidated, demanding further exploration and validation in larger cohorts. The ongoing investigation into the clinical impact of these mutations in our study aligns with the broader effort to comprehensively understand the prognostic implications of molecular profiles in OCCC [26,27]. Our research presents a novel method for categorizing OCCC through prevalent mutational signatures, elucidating the activation of the C-APOBEC enzyme and the impact of aging processes. This alternative classification augments the traditional genetic profiling, offering a more comprehensive understanding of the genomic landscape in OCCC. The proposed subgroups hold potential implications for treatment strategies and prognostic assessments, warranting future investigations to validate their clinical relevance and impact on patient outcomes. Comparatively, existing literature supports the notion that mutational signatures, such as APOBEC family signatures, are associated with specific characteristics in ovarian clear cell carcinoma. The identification of these signatures can contribute to a deeper comprehension of the underlying molecular mechanisms, potentially aiding in the development of targeted therapies and personalized treatment approaches [28,29]. A notable revelation from our study is the exploration of Tumor Mutational Burden (TMB) in OCCC. Calculating TMB, reflective of the total number of mutations per megabase, unveils a promising avenue for prognostic assessment in OCCC. TMB has emerged as a prognostic indicator and a putative biomarker for immunotherapy across various cancer types. However, its specific relevance and prognostic impact in the context of OCCC remain insufficiently explored. Our study sets the stage for future investigations into the intricate relationship between TMB, treatment response, and overall survival in OCCC. Existing literature supports the broader significance of TMB in predicting treatment outcomes and overall survival in various cancers. While our study emphasizes the untapped potential of TMB in OCCC, it aligns with the broader trend recognizing TMB as a valuable prognostic tool [29].

Limitations and Future Directions

Acknowledging the strengths and limitations of our study is crucial for contextualizing the findings. The retrospective nature of the analysis and the absence of functional assays to validate the biological impact of identified mutations represent inherent constraints. The relatively modest sample size, though carefully curated, necessitates prudence in extrapolating findings to the broader OCCC population.

Future research endeavors should prioritize elucidating the functional consequences of ARID1A and PIK3CA mutations, exploring their potential as therapeutic targets, and validating the proposed molecular subgrouping based on mutational signatures in larger, prospective cohorts. Comprehensive studies, incorporating a multidimensional approach, would contribute to refining our understanding of the molecular landscape in OCCC and inform more personalized treatment strategies.

Conclusion

In conclusion, our study significantly advances the understanding of OCCC's molecular intricacies, providing a robust foundation for future research endeavors. The molecular heterogeneity uncovered, the potential clinical implications of ARID1A and PIK3CA mutations, alternative subgrouping based

on mutational signatures, and the exploration of TMB as a prognostic indicator collectively contribute to the evolving narrative of OCCC. As the field progresses, these insights hold promise for informing targeted therapies and personalized treatment strategies, ultimately enhancing outcomes for patients diagnosed with this distinctive ovarian cancer subtype.

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