Integration of Molecular and Histopathologic Classification: A Pathway to Optimize Treatment and Prognosis in Endometrial Cancer

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Integração da Classificação Molecular e Histopatológica: Um Caminho para Otimizar o Tratamento e o Prognóstico no Câncer de Endométrio

Integración de la Clasificación Molecular e Histopatológica: Un Camino para Optimizar el Tratamiento y el Pronóstico en el Cáncer de Endometrio

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ABSTRACT

Introduction: Endometrial cancer, the sixth most common neoplasm in postmenopausal women, is experiencing a rising global incidence. Traditionally, it is classified into type I (endometrioid) and type II (non-endometrioid), and more recently into four molecular groups with different prognostic and therapeutic implications. **Objective:** To analyze the impact of integrating histopathology and molecular biology on the understanding of EC and its influence on treatment and patient prognosis. **Method:** A retrospective study of 1,451 EC cases from The Cancer Genome Atlas (TCGA) project, using ANOVA, t-tests, and chi-square tests for genomic and clinical evaluation. **Results:** Endometrioid carcinoma was predominant, with a mean age at diagnosis of 63.7 years and variations in overall survival by race. Adjuvant radiotherapy was effective in patients with endometrioid carcinoma, with an overall survival of 35.4 months. Neoadjuvant therapy significantly improved overall survival in uterine papillary serous carcinomas. 87% of patients with endometrioid carcinoma, 48% with mixed endometrial carcinoma, and 25% with undifferentiated endometrial carcinoma benefited from detailed molecular analysis. The molecular groups showed distinct gene expression characteristics. Group 1 suggested dynamic disease control. Group 2 was dominated by genes related to the cell cycle and chromatin stability. Group 3 highlighted genes involved in chromatin remodeling and cell proliferation, while Group 4 was characterized by TP53 expression and genes related to the PI3K-AKT pathway. **Conclusion:** Given the molecular complexity of endometrial cancer, integrating histopathological and molecular analyses is essential for guiding precise treatments, increasing therapeutic efficacy, and improving clinical outcomes.

Key words: Endometrial Neoplasms; Postmenopause; Cytogenetic Analysis/methods; Precision Medicine/methods.

RESUMO

Introdução: O câncer de endométrio, sexta neoplasia mais comum em mulheres pós-menopausa, apresenta incidência crescente globalmente. Tradicionalmente, é classificado em tipos I (endometrioide) e II (não endometrioide) e, mais recentemente, em quatro grupos moleculares, com diferentes implicações prognósticas e terapêuticas. **Objetivo:** Analisar o impacto da integração entre histopatologia e biologia molecular na compreensão do câncer de endométrio e sua influência no tratamento e prognóstico das pacientes. **Método:** Estudo retrospectivo de 1.451 casos do projeto *The Cancer Genome Atlas* (TCGA), utilizando ANOVA, teste *t* e qui-quadrado para avaliação genômica e clínica. **Resultados:** O carcinoma endometrioide foi predominante, com média de idade ao diagnóstico de 63,7 anos e sobrevida global variando por raça. A radioterapia adjuvante mostrou-se eficaz em pacientes com carcinoma endometrioide, com sobrevida global de 35,4 meses. A terapia neoadjuvante aumentou significativamente a sobrevida global em carcinomas serosos papilares uterinos. Puderam se beneficiar da análise molecular 87% das pacientes com carcinoma endometrioide, 48% com carcinoma misto endometrial e 25% com carcinoma endometrial indiferenciado. Os grupos moleculares apresentaram características distintas de expressão gênica. As do Grupo 1 sugeriram controle dinâmico da doença. No Grupo 2, predominaram genes ligados ao ciclo celular e estabilidade da cromatina. O Grupo 3 destacou genes envolvidos no remodelamento da cromatina e proliferação celular, enquanto o Grupo 4 caracterizou-se pela expressão de TP53 e de genes relacionados à via PI3K-AKT. **Conclusão:** Dada a complexidade molecular do câncer de endométrio, integrar análises histopatológicas e moleculares é essencial para guiar tratamentos precisos, aumentando a eficácia terapêutica e melhorando desfechos clínicos.

Palavras-chave: Neoplasias do Endométrio; Pós-Menopausa; Análise Citogenética/métodos; Medicina de Precisão/métodos.

RESUMEN

Introducción: El cáncer de endometrio, sexta neoplasia más común en mujeres posmenopáusicas, presenta una incidencia creciente globalmente. Se clasifica en tipos I (endometrioide) y II (no endometrioide), y más recientemente en cuatro grupos moleculares, con diferentes implicaciones pronósticas y terapéuticas. **Objetivo:** Analizar el impacto de la integración entre histopatología y biología molecular en la comprensión del cáncer de endometrio y su influencia en el tratamiento y pronóstico de las pacientes. **Método:** Estudio retrospectivo de 1451 casos del proyecto *The Cancer Genome Atlas* (TCGA), utilizando ANVA, pruebas *t* y ji al cuadrado para evaluación genómica y clínica. **Resultados:** El carcinoma endometrioide fue predominante, con media de edad al diagnóstico de 63,7 años y supervivencia global variable según la raza. La radioterapia adyuvante fue eficaz en pacientes con carcinoma endometrioide, con supervivencia global de 35,4 meses. La terapia neoadyuvante aumentó significativamente la supervivencia global en carcinomas serosos papilares uterinos. El 87% de las pacientes con carcinoma endometrioide, el 48% con carcinoma mixto endometrial y el 25% con carcinoma endometrial indiferenciado pudieron beneficiarse del análisis molecular. Los grupos moleculares presentaron características de expresión génica. Las del Grupo 1 sugirieron control dinámico de la enfermedad. En el Grupo 2 predominaron los genes relacionados con el ciclo celular y estabilidad de la cromatina. El Grupo 3 destacó genes involucrados en remodelado de la cromatina y proliferación celular, mientras que el Grupo 4 se caracterizó por expresión de TP53 y genes relacionados con la vía PI3K-AKT. **Conclusión:** Debido a la complejidad molecular del cáncer de endometrio, integrar análisis histopatológicos y moleculares es esencial para guiar tratamientos precisos, aumentando la eficacia terapéutica y mejorando resultados clínicos.

Palabras clave: Neoplasias del Endometrio; Posmenopausia; Análisis Citogenético/métodos; Medicina de Precisión/métodos.

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INTRODUCTION

Uterine cancer can originate in the endometrium (90% of cases) or in the myometrium[1]. It is the sixth most common neoplasm among women in menopause worldwide, with a rising incidence proportional to the Human Development Index. The highest incidence rates have been recorded in North America and Europe[2,3]. In Brazil, disregarding non-melanoma skin cancer, uterine cancer is the seventh most common cancer in women. The INCA estimates for the 2023-2025 triennium projected 9,650 new cases per year, with a rate of 5.22 new cases for every 100 thousand women[4]. In 2023, the crude mortality rate attributed to this neoplasm was 6.65 deaths per 100 thousand women, according to data from INCA's online cancer mortality atlas[5].

The main risk factors for endometrial cancer include prolonged exposure to estrogen, obesity, diabetes mellitus (DM), systemic arterial hypertension (SAH), endometrial hyperplasia, chronic anovulation, radiation for ovarian tumors, hormone replacement therapy, early menarche, late menopause, Polycystic Ovary Syndrome, and Lynch Syndrome.[2,5].

Classically, endometrial cancers are classified into two subtypes: type I (endometrioid), estrogen-dependent and usually associated with favorable outcomes, and type II (non-endometrioid), which includes serous, mucinous, or clear cell tumors, with a worse prognosis[6,7]. Endometrial cancer staging is surgical and based on anatomopathological findings. Molecular classification into four main groups has important prognostic and therapeutic implications, enabling more precise risk stratification[8].

In this context, the World Health Organization (WHO) issued in 2020 a recommendation to include molecular aspects in the pathological reports whenever possible[8]. It is worth highlighting that histopathological characteristics did not lose their role in diagnosis; however, the relative relevance of these characteristics in making therapeutic decisions varies according to molecular subtype. Additionally, the prognostic value of molecular classification has been consistently confirmed by cohort studies and clinical trials[8,9]. With all that considered, this study aimed to analyze the impact of integrating histopathology and molecular biology to understand endometrial cancer and its influence on treatment and patient prognosis.

METHOD

This study was designed with a retrospective, exploratory, and analytical profile, using genomic and clinical data available from the *cBioPortal for Cancer Genomics*[10].

Initially, 2,115 uterine cancer cases were selected. After refinement, 1,451 cases from the "The Cancer Genome Atlas (TCGA) Uterine Corpus Endometrial Carcinoma" project remained, encompassing three studies: 549 cases from the "GDAC Firehose", 373 cases from the "TCGA, Nature 2013", and 529 cases from the "TCGA, PanCancer Atlas". The inclusion criteria considered primary endometrial cancer, excluding 607 cases of cervical squamous cell carcinoma and 114 carcinosarcoma cases. Due to the independent nature of the studies and the inconsistent availability of clinical information, not all analyses could be performed across the 1,451 selected cases.

The target population includes patients with endometrial cancer whose clinical and genomic data were available. The collected information included: age at diagnosis, menopause status, histopathological tumor type, surgical staging, survival, history of previous malignant neoplasms, synchronous malignant neoplasms, body mass index (BMI), use of hormonal contraceptives, comorbidities, including SAH and DM, undergone treatments, and gene expression data. Menopause status was defined according to WHO criteria, which considered pre-menopause patients those with regular menstrual cycles, climacteric those who were transitioning to menopause, and patients with amenorrhea for at least 12 months or more, not attributed to other causes, were considered post-menopause. The clinical, genomic data, and referring to overall survival and disease-free survival were obtained and enriched through the *cBioPortal for Cancer Genomics*[10] and *Bioinformatics & Evolutionary Genomics*[11] platforms.

Variance analysis (ANOVA), followed by Tukey's multiple comparisons test[12], or *t* test[13], was conducted using the GraphPad Prism[12] software, version 6.01 (GraphPad Software, San Diego, CA, USA). To assess equality of proportions between the four molecular groups of endometrial cancer, chi-square tests[14] were conducted using the *prop.test* function in R software[15], version 4.3.0. For every analysis, a significance level of $p<0.05$ was adopted.

As this was an *in silico* analysis, this study required no approval from the Research Ethics Committee, in compliance with Resolution number 510/2016 of the National Health Council[16].

RESULTS

Endometrioid carcinoma was the most common histopathological type (1,116 cases), followed by uterine

papillary serous carcinoma (277 cases), undifferentiated endometrial carcinoma (37 cases), and mixed endometrial carcinoma (21 cases).

The mean age at endometrial cancer diagnosis was 63.7 years, with the age group ranging from 31 to 90 years. Regarding race, eight cases were identified in American Indian or Alaskan Native women, 40 cases in Asian women, 216 cases in black, African American women, 18 cases in Native Hawaiian or other Pacific Islander, and 732 cases in white women. The average ages at diagnosis varied between racial groups: 54.2 years for Native Hawaiian or other Pacific Islander, 54.3 years for Asians, 61 years for American Indian or Alaskan Native, 64.4 years for white women, and 65 years for black/African American women (Table 1).

Data on overall survival revealed significant differences between some racial groups. American Indian or Alaskan Native (26.2 months), black/African American (32.7 months), and white women (37.2 months) had significantly lower survival rates than Asian (62.0 months) and Native Hawaiian or other Pacific Islander women (54.3 months) (Table 1).

Regarding age group, 35 cases were in pre-menopause, 17 cases in the climacteric period, and 449 cases were in menopause.

In regard to endometrial cancer risk factors, an average BMI of 33.8 was found for the 520 cases with available information on weight and height. As to hormonal therapy, one case reported the use in menopause, and seven cases claimed the use of hormonal contraceptives, while 21 cases never used it. The analysis further revealed a patient with a history of colorectal cancer. Moreover, 31 patients presented a history of SAH, and 15 presented a history of DM.

The histopathological characterization showed a predominance of endometrioid carcinoma, corresponding to 76.9% of the analyzed cases. The other subtypes included uterine papillary serous carcinoma (19.1%), undifferentiated endometrial carcinoma (2.5%), and mixed endometrial carcinoma (1.4%) (Table 1).

Regarding histopathological staging according to the International Federation of Gynaecology and Obstetrics (FIGO[17] 1988, revised in 2009), stage I was the most common (23.6%), presenting an overall average survival of 39.6 months. Stages II, III, and IV corresponded to 3.6%, 8.5%, and 2.1% of cases, with average survivals of 39.7, 32.8, and 29.6 months, respectively. There was a high proportion of cases with uninformed staging (62.2%), whose overall average survival was 34.9 months (Table 1).

Regarding the FIGO histopathological grade, most samples were classified as grade 3 (G3), representing 42.7% of cases, with an overall average survival of 36.4 months. Grades G2 and G1 corresponded to 16.6% and 13.5% of cases, with average survivals of 36.2 and 43.9 months, respectively, with statistically significant differences observed between groups (Table 1).

Surgery constitutes the main initial therapeutic approach in endometrial cancer and can be associated with adjuvant or neoadjuvant therapies according to staging, histopathological subtype, and patients' clinical conditions. In this study, adjuvant radiotherapy was significantly effective in patients with endometrioid carcinoma, being associated with greater overall survival (35.4 months) when compared to patients who did not receive this therapeutic modality (11.8 months) (Table 2).

In uterine papillary serous carcinomas, neoadjuvant therapy significantly increased overall survival, indicating greater efficacy in this histopathological subtype (Table 2). However, this analysis must be deepened, since the data refers to only two patients, which requires cautious interpretation. Additionally, histopathological subtypes represented by only one patient were not submitted to statistical analysis due to the statistical impossibility of performing inferential tests with a single sample.

All the samples were initially classified by histopathological subtypes and, within each subtype, the percentages corresponding to each genomic group were identified: Group 1 (POLE), Group 2 (Microsatellites Instability – MSI), Group 3 (Copy-number Low), and Group 4 (Copy-number High) (Figure 1A).

The analysis highlighted the expressive molecular heterogeneity within the endometrial cancer histopathological subtypes. In the endometrioid carcinoma samples, 87% of cases were distributed in different molecular groups, indicating a wide genomic variability within that subtype (Figure 1A). Among the mixed endometrioid carcinomas, approximately 48% of the samples presented distribution in multiple molecular subgroups, stressing the biological diversity of this group (Figure 1A). Whereas in uterine papillary serous carcinomas, 94% of the samples have genomic characteristics distinct from the previous groups, directly influencing therapeutic decisions (Figure 1A).

The detailed analysis of the four molecular endometrial cancer groups revealed shared and distinct characteristics of gene expression, underscoring molecular digital impressions. From the analysis, 19,205 expressed genes were identified, of which: 18,957 were related to Group 1, 18,116 to Group 2, 6,932 to Group 3, and 7,263 to Group 4 (Figure 1B). Of that total, 1,132 are classified as Cancer Genes according to the OncoKB™ Cancer Gene List[18], which were subdivided according to the molecular groups to which they belong (Figure 1C).

In Group 1, genes POLE, FAT4, PTEN, PCLO, CCNB3, ATRX, MKI67, REV3L, LRP1B, and KMT2C

Table 1. Clinical aspects, diagnosis, and FIGO staging of endometrial cancer

Clinical Data	Characteristics	N# of cases	Average age at diagnosis [MIN;MAX]	Overall average survival (months)
General age at diagnosis	Endometrial Cancer	1,445 (99.5%)	63.7 [31;90]	35.9
Race	American Indian or Alaskan Native	8	61.0 [51;77]	26.2[a]
	Asian	40	54.3 [31;69]	62.0[abc]
	Black or African American	216	65.0 [33;87]	32.7[bd]
	Native Hawaiian or other Pacific Islander	18	54.2 [39;68]	54.3[d]
	White	732	64.4 [33;90]	37.2[c]
Primary Histopathological Diagnosis	Endometrioid carcinoma	1,116 (76.9%)	62.3 [31;90]	36.8
	Uterine papillary serous carcinoma	277 (19.1%)	68.7 [45;90]	34.3
	Undifferentiated endometrial carcinoma NOS	37 (2.5%)	66.4 [47;83]	25.5
	Mixed endometrial carcinoma	21 (1.4%)	66.4 [47;83]	29
Histopathological staging – FIGO, 1988, reviewed in 2009	I	343 (23.6%)		39.6
	II	52 (3.6%)		39.7
	III	124 (8.5%)		32.8
	IV	30 (2.1%)		29.6
	Not informed	902 (62.2%)		34.9
FIGO histopathological grade	G1	196 (13.5%)	-	43.9[ef]
	G2	241 (16.6%)	-	36.2[e]
	G3	619 (42.7%)	-	36.4[f]

Captions: Equal letters ([a], [b], [c], [d], [e], or [f]) in different lines indicate that the means in the respective groups statistically differ among them, according to Tukey's multiple comparisons test (p<0.05). FIGO: International Federation of Gynaecology and Obstetrics. NOS: not otherwise specified.

stood out as the 10 most frequently expressed in the evaluated samples (Figure 1D). Moreover, 21 Cancer Genes exclusively expressed in this group were identified (Figure 1C). Among the tumoral suppressors, WIF1 (30.3% of cases), HTATIP2 (22.7%), BTG2 (8%), and KLF2 (4.1%) were noticed. On the other hand, oncogenes TNFSF13 (11.8%), CKS1B (7.6%), TLX1 (7.6%), FGF4 (2%), and FOXL2 (2%) stood out. Genes like HOXD13 (21.3%), TAL1 (7.6%), BTG1 (6.1%), and HMGA2 (2%) were also observed, associated with gene transcription regulation, in addition to CENPA (6.1%), H3C8 (5.9%), RPS15 (5.9%), and TCL1A (4.5%), related to genomic stability, cell cycle control, and cell survival. Moreover, the genes SSX4 (15.2%), CD79B (15.2%), MDS2 (2%), and BAALC (12.1%), associated with immune response modulation, myelodysplastic syndromes, and synaptic functions, respectively, were also observed.

Group 1 represented 8.9% of evaluated cases, with an average age at diagnosis of 56.6 years and an overall survival of 49 months, significantly higher than that observed in other groups (p<0.01) (Figures 1E and 1F). Disease-free survival was 89.8% in 12 months, 73.5% in 24 months, 59.2% in 36 months, 46.9% at 48 months, and 34.7% at 60 months, with statistically significant differences at 36, 48, and 60 months (p<0.05), in relation to the other groups (Figures 1E and 1G).

In Group 2, the gene expression profile revealed that genes PTEN, ARID1A, PIK3CA, KMT2D, KMT2B, ZFHX3, PIK3R1, CTCF, RPL22, and KRAS were the 10 most frequently expressed in the assessed samples (Figure 1D). Additionally, six genes were exclusively expressed in this group: H4C9 (3.1% of cases), H3C1 (1.5%), H2BC17 (1.5%), CDKN2B (1.4%), CEBPA (0.7%), and MTCP1 (0.7%) (Figure 1C). Genes H3C1, H4C9, and H2BC17 codified histones, performing essential roles in DNA organization, transcription, replication, and repair. Genes CDKN2B, CEBPA, and MTCP1 are implicated in the cycle regulation and cell differentiation. CDKN2B

Table 2. Adjuvant and neoadjuvant treatments in endometrial cancer per histopathological subtype

Treatment	Endometrial Cancer Histopathological Subtype	N# of cases		Overall average survival (months)	
		No	Yes	No	Yes
Radiotherapy	Endometrioid carcinoma	227	156	40.6	39.2
	Uterine serous carcinoma/Uterine papillary serous carcinoma	47	53	40.2	33.2
	Endometrial carcinoma	-	-	-	-
	Mixed uterine endometrial carcinoma	10	10	27.6	28.4
Adjuvant radiotherapy	Endometrioid carcinoma	12	17	11.8	35.4*
	Uterine serous carcinoma/Uterine papillary serous carcinoma	6	11	21.3	21.7
	Endometrial carcinoma	1	3	18.4	33.3
	Mixed uterine endometrial carcinoma	-	-	-	-
Adjuvant chemotherapy	Endometrioid carcinoma	21	8	27.2	21.7
	Uterine serous carcinoma/Uterine papillary serous carcinoma	5	12	21.9	21.4
	Endometrial carcinoma	1	3	41	25.7
	Mixed uterine endometrial carcinoma	-	-	-	-
Neoadjuvant Therapy	Endometrioid carcinoma	808	1	39	27.1
	Uterine serous carcinoma/Uterine papillary serous carcinoma	222	2	34	80.4*
	Endometrial carcinoma	24	-	29.3	-
	Mixed uterine endometrial carcinoma	21	-	29	-

Caption: *Indicates statistically significant difference (p<0.05) in the overall average survival between patients who received treatment or not, according to Student's t test.

codifies p15 protein, which inhibits cyclin-dependent kinases (CDK) in the transition from G1 phase to S phase. CEBPA acts like an essential transcription factor for the differentiation of hematopoietic cells, while MTCP1 is involved in intracellular signaling that promotes survival and proliferation of T cells. This group corresponded to 28.65% of cases, with an average age at diagnosis of 63 years and overall survival of 35.96 months (Figures 1E and 1F).

Group 3 frequently presented expression of the genes PTEN, PIK3CA, CTNNB1, ARID1A, PIK3R1, CTCF, KRAS, FGFR2, CHD4, and SPOP (Figure 1D). Moreover, this group shared with Group 1 the expression of genes IGF1 (45.5% in Group 1 and 0.8% in Group 2), IL2 (9.1% in Group 1 and 0.8% in Group 2), and JUN (9.1% in Group 1 and 0.7% in Group 2), involved in the signaling of growth, immune response, and transcription regulation, respectively. Additionally, Group 3 shared with Groups 1 and 4 the expression of H2AC17 (5.9% in Group 1, 1.1% in Group 3, and 3.3 in Group 4), a variant of H2A Histone, which can modify the structure of chromatin and gene expression. Thus, the analysis of gene expression in Group 3 revealed a complex combination of genes involved in critical processes, like

chromatin remodeling, cellular signaling, adherence, and proliferation, which can influence both disease progression and response to treatment. Group 3 represented 32.03% of evaluated cases, with an average age at diagnosis of 61.1 years and an overall survival of 34.86 months (Figures 1E and 1F).

Group 4 had TP53, PIK3CA, PPP2R1A, FBXW7, PTEN, CHD4, PIK3R1, ARHGAP35, SPOP, and TAF1 among their 10 most expressed genes (Figure 1C). This group also shared with Group 2 the expression of genes ID1 (1.9% in Group 2 and 0.6% in Group 4), and H3C13 (1.4% in Group 2 and 0.9% in Group 4). Gene ID1 regulates cellular senescence, growth, and cell survival. Gene H3C13 codifies a histone of the H3 family, essential to chromosome condensation, and alterations in this dynamic are associated with malignant transformation. Group 4 encompassed 30.13% of evaluated cases, with an average age at diagnosis of 68.8 years and an overall survival of 34.86 months (Figures 1E and F).

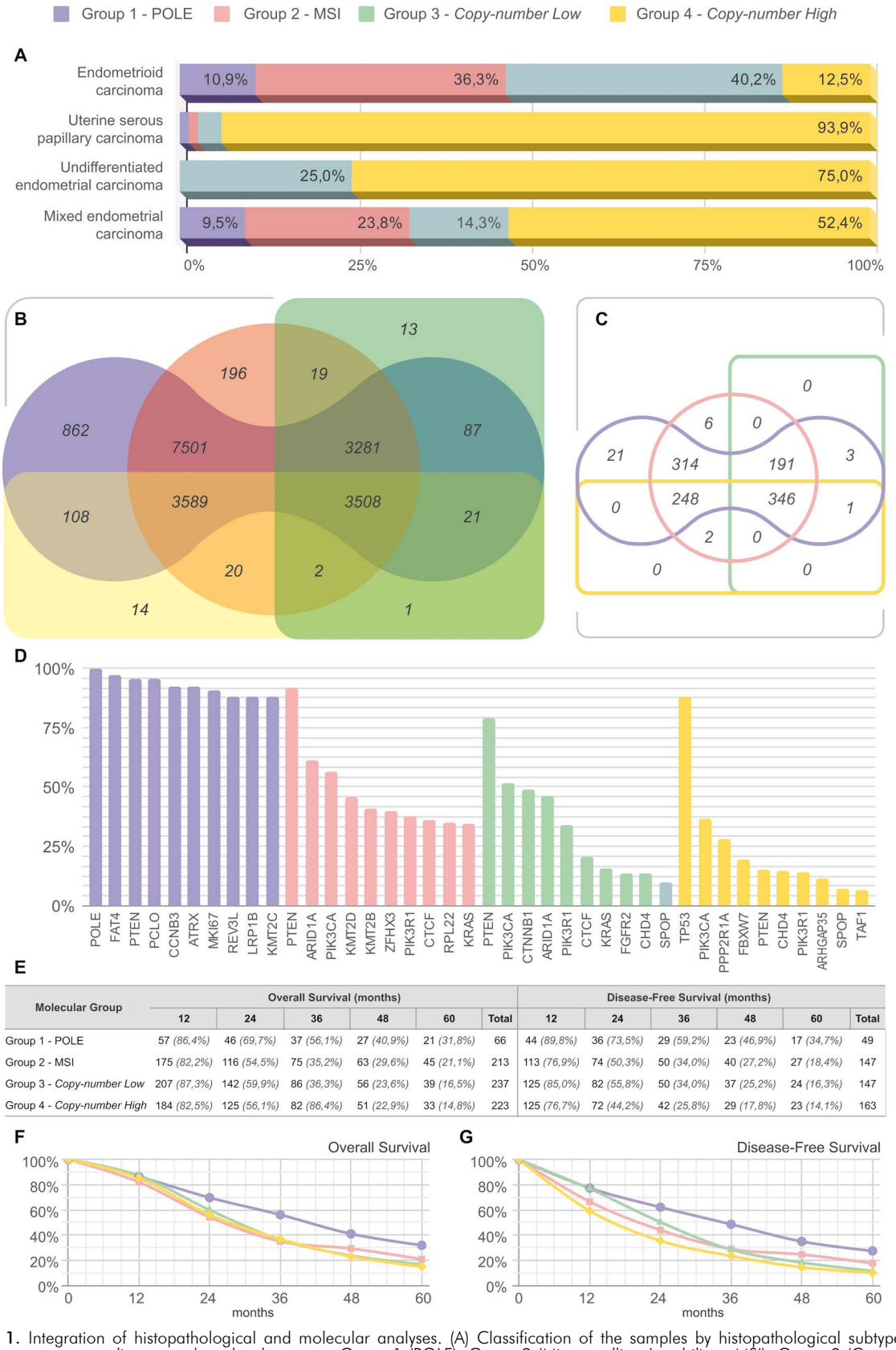
DISCUSSION

The integration of morphological and molecular analyses is key to optimizing the treatment of endometrial cancer. One of the main current challenges is to differentiate patients with a high risk of recurrence, who need more aggressive therapeutic approaches, from those who can benefit from more conservative strategies. Classically, risk stratification is based on morphological characteristics, which often have low reproducibility, especially in high-grade histological subtypes[19]. This diagnostic limitation can lead to the inclusion of biologically diverse tumors in the same group, impairing therapeutic efficacy. In this context, in 2020, the WHO recommended including molecular aspects in pathological reports[3].

The analysis of clinical and epidemiological characteristics of patients with endometrial cancer included in this study demonstrated that endometrioid carcinoma is the most prevalent type, corresponding to 76.9% of cases, and predominant in white women with an average age at diagnosis of 63.7 years, corroborating data from previous studies[9]. The disparities in overall survival observed between racial groups suggested that racial-ethnic and socioeconomic factors play an important role in disease evolution. Although Asian and Hawaiian native and other Pacific Islander women have been diagnosed at similar ages, Asians presented significantly higher survival rates. Additionally, most cases occurred in women in menopause with high average BMI, reinforcing the already well-established in the literature association between obesity and increased risk of endometrial cancer[20].

Genetic screening of endometrial cancer plays a crucial role in the identification of high-risk patients and customization of treatment strategies. In this context, this study reinforced the importance of an integrated approach between histopathology and molecular biology, a widely acknowledged concept, but still evolving in clinical practice. The identification of molecular subgroups within each histopathological subtype, as highlighted in this work, revealed critical nuances that may be lost in traditional classification. In the case of endometrioid carcinoma samples, 87% of patients benefited from their molecular subtype being known, through more effective and less toxic treatments. For the assessed mixed endometrial carcinomas, identification of molecular subgroups is equally crucial, improving therapeutic options for approximately 48% of patients. Therefore, molecular classification not only complemented but improved the prediction of tumoral behavior and the management of endometrial cancer, from the choice of treatment to improvement of patients' prognoses. Particularly, the ability to individualize the treatment underscores the need for a more precise and less toxic approach, as suggested by molecular-based studies — especially for patients with uterine papillary serous carcinomas, whose molecular classification indicated distinct genomic characteristics in 94% of cases[8,9,20].

The Group 1 analysis revealed POLE, FAT4, PTEN, PCLO, CCNB3, ATRX, MKI67, REV3L, LRP1B, and KMT2C as the most frequent genes in the assessed samples, which shows the molecular complexity of endometrial cancer. Among the 21 genes exclusively expressed in this group, tumoral suppressors, like WIF1 (30.3%), HTATIP2 (22.7%), BTG2 (8%), and KLF2 (4.1%) were predominant, concomitant to the presence of oncogenes, including TNFSF13 (11.8%), CKS1B (7.6%), and TLX1 (7.6%). This expression pattern suggests the coexistence of a complex interaction between suppression mechanisms and tumoral promotion, reflecting a possible dynamic molecular regulation mechanism during disease progression. The detection of genes related to transcription regulation, like HOXD13 (21.3%) and TAL1 (7.6%), and to genomic stability, like CENPA (6.1%) and H3C8 (5.9%), reinforced the idea that these genetic alterations play a critical role in endometrial cancer progression[21]. Additionally, the presence of genes like SSX4 (15.2%) and CD79B (15.2%), associated with immune response, suggested a possible influence in tumoral interaction with the micro-environment[22,23]. Despite the observed functional heterogeneity, Group 1 stood out for the significant expression of tumoral suppressor genes and the superiority in terms of overall survival and disease-free survival in comparison to other



Molecular Group	Overall Survival (months)						Disease-Free Survival (months)					
	12	24	36	48	60	Total	12	24	36	48	60	Total
Group 1 - POLE	57 *(86,4%)*	46 *(69,7%)*	37 *(56,1%)*	27 *(40,9%)*	21 *(31,8%)*	66	44 *(89,8%)*	36 *(73,5%)*	29 *(59,2%)*	23 *(46,9%)*	17 *(34,7%)*	49
Group 2 - MSI	175 *(82,2%)*	116 *(54,5%)*	75 *(35,2%)*	63 *(29,6%)*	45 *(21,1%)*	213	113 *(76,9%)*	74 *(50,3%)*	50 *(34,0%)*	40 *(27,2%)*	27 *(18,4%)*	147
Group 3 - *Copy-number Low*	207 *(87,3%)*	142 *(59,9%)*	86 *(36,3%)*	56 *(23,6%)*	39 *(16,5%)*	237	125 *(85,0%)*	82 *(55,8%)*	50 *(34,0%)*	37 *(25,2%)*	24 *(16,3%)*	147
Group 4 - *Copy-number High*	184 *(82,5%)*	125 *(56,1%)*	82 *(86,4%)*	51 *(22,9%)*	33 *(14,8%)*	223	125 *(76,7%)*	72 *(44,2%)*	42 *(25,8%)*	29 *(17,8%)*	23 *(14,1%)*	163

Figure 1. Integration of histopathological and molecular analyses. (A) Classification of the samples by histopathological subtypes, with percentages corresponding to each molecular group: Group 1 (POLE), Group 2 (Microsatellites Instability – MSI), Group 3 (Copy-number Low), and Group 4 (Copy-number High, or high alteration in the number of copies). (B) Venn diagram illustrating the most frequently mutated genes in endometrial cancer, based on the mutational data analysis available on *cBioPortal*. (C) Venn diagram exhibiting the genes associated with cancer, according to the OncoKB™ Cancer Gene List, subdivided into corresponding molecular groups. (D) The 10 most frequently expressed genes in the assessed samples, subdivided by molecular groups. (E and F) Analysis of overall survival by molecular group. (G) Analysis of disease-free survival by molecular group.

groups. These findings reinforce the prognostic relevance of molecular classification and underscore the importance of incorporating this information in the development of more precise and effective therapeutic strategies.

The analysis of Groups 2, 3, and 4 revealed distinct patterns of gene expression and their implications for endometrial cancer. In Group 2, mutations in histones stood out, like H3C1 (1.5%), H4C9 (3.1%), and H2BC17 (1.5%), which showed the destabilization of chromatin. These findings were consistent with the literature, which frequently records mutations in histones in endometrial cancer, especially in the H2A[24], H2B[25], H4, and H3[26] families. Moreover, genes CDKN2B (1.4%), CEBPA (0.7%), and MTCP1 (0.7%), which regulate the cellular differentiation cycle, were shown to be relevant in the analyzed samples, corroborating the functional role previously attributed to these genes in the oncological context[27-29].

Group 3 presented frequent expression of the genes PTEN, PIK3CA, CTNNB1, ARID1A, PIK3R1, CTCF, KRAS, FGFR2, CHD4, and SPOP, implicated in fundamental processes to endometrial cancer, like cellular signaling, chromatin remodeling, and cellular adherence. This group also shared with Group 1 the expression of genes IGF1, IL2, and JUN, which are known to play roles in signaling growth [30], immune response[31], and transcription regulation[32]. The expression of H2AC17, a histone variant shared by Groups 1, 3, and 4, seems to suggest that modulation of chromatin structure and gene expression regulation are potentially implicated in endometrial cancer progression[33]. Group 3 corresponded to 32.03% of cases, with an average age at diagnosis of 61.1 years and an overall survival of 34.86 months. The combination of these genes highlighted the molecular complexity of Group 3 and pointed to potential therapeutic targets, given its influence both in disease progression as well as in treatment response.

Group 4 presented a notorious expression of TP53 (87.9% of samples), a fundamental gene in the response to genetic damage, in addition to genes involved in the signaling route PI3K/AKT, like PIK3CA, PIK3R1, and PTEN, which regulate survival and cellular proliferation[33,34]. The expression of PPP2R1A, associated with response to cellular homeostasis and stress, and of FBXW7, a tumoral suppressor responsible for degrading oncoproteins, also stood out. Other important genes included CHD4 and TAF1, involved in chromatin remodeling and gene transcription regulation, in addition to ARHGAP35, which influences cellular migration and adherence through cytoskeleton regulation, and SPOP, involved in ubiquitination and protein degradation. This group shared with Group 2 the expression of ID1 and H3C13, genes associated with the regulation of cellular senescence, growth, and survival, as well as chromosomal condensation, processes that are frequently altered in malignancies[26]. Comprising 30.13% of cases, Group 4 presented an average age at diagnosis of 68.8 years and overall survival of 34.86 months.

The Brazilian legislation advance in oncology treatment is undeniable, but there is still room for improvement. Law N. 12,732/2012[35] ensures that referrals and access to oncology therapies are continually updated, ensuring the implementation of new proven treatments and the right to start them within 60 days. In this scenario, precision medicine has also progressed, although significant challenges persist. Resolution N. 340/2004[36] and Ordinance N. 81/2009[37] of the Ministry of Health establish guidelines for clinical genetics and integral care policies, respectively. However, access to precision medicine in Brazil is predominantly restricted to private institutions. The National Health System (SUS) offers limited coverage, mainly due to budget and infrastructure restrictions. The utilization guidelines for coverage of procedures in supplementary healthcare, established by Normative Resolution N. 465/2021[38] of the National Supplementary Health Agency, represent a relevant advancement in establishing the coverage of tests for conditions associated with specific genetic mutations, like Cowden Syndrome and Lynch Syndrome. This coverage includes patients and their first, second, or third-degree relatives, reflecting progress in the inclusion of precision medicine practices.

In summary, to translate legislative and scientific advancements into widely accessible benefits to the population, it is fundamental to strengthen SUS with continuous investments in infrastructure, professional training, and updates of public healthcare policies, to ensure that every patient, regardless of their social class, may benefit from therapeutic innovations. In this context, the findings of this study, grounded in the integration of clinical, histopathological, and molecular data from a wide TCGA cohort, reinforce the precision medicine potential to improve oncological care in endometrial cancer. We are aware of the limitations inherent to the retrospective design, such as the possibility of selection bias and incompleteness of certain clinical variables, as well as a possible temporal bias, considering that data from the TCGA may not completely reflect the most recent advancements in targeted therapies and immunotherapy. Additionally, the results related to undifferentiated endometrial carcinoma and mixed endometrial carcinoma must be interpreted with caution due to the reduced number of cases. Nevertheless, the results presented are a relevant contribution to understanding endometrial cancer heterogeneity and support the need for increasing access to customized diagnostic and therapeutic strategies.

CONCLUSION

This study highlights the fundamental role of integrating histopathology and molecular biology into the understanding and treatment of endometrial cancer. The identification of molecular subtype would benefit 87% of endometrioid carcinoma patients, enabling more effective treatments with fewer side effects. In mixed carcinomas, the molecular analysis would improve therapeutic options for approximately 48% of patients. Molecular classification, therefore, not only complements but increases the ability to predict tumoral behavior and guide the clinical management of endometrial cancer. This is particularly evident in uterine papillary serous carcinomas, in which 94% of samples presented significant genomic variations, reinforcing the need for more precise and less toxic therapeutic approaches.

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Eliane Gouvêa de Oliveira Barros and Guilherme Tavares Cruz have contributed to the study design, analysis, and interpretation of the data, wording, and critical review. Maria Bethânia Souza Vilela, Gabriela Silva Castro, Jéssica Menezes do Nascimento, Larissa Marques Gonçalves and Olga Maria Silva Castro have contributed to data interpretation. Anna Marcella Neves Dias, Nathália Barbosa do Espírito Santo Mendes, and Bruno Aquino Marcelino contributed to the critical review. All the authors approved the final version for publication.

DECLARATION OF CONFLICT OF INTERESTS

There is no conflict of interest to declare.

DATA AVAILABILITY STATEMENT

The crude datasets generated and analyzed in this article are available upon request to the corresponding author and may be publicly accessed through the *cBioPortal for Cancer Genomics* platform, available at: https://www.cbioportal.org/study?id=697278afae9c9e25baed2c39

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