Profesional paper

CANCER STEM CELL RELATED MARKER CD44 IN LOW GRADE ENDOMETRIAL CANCER

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ABSTRACT

Background: Endometrial cancer (EC) is the most prevalent gynecological malignancy, traditionally classified into type 1 (estrogen-dependent, low-grade) and type 2 (high-grade, aggressive).While histological parameters serve as prognostic indicators, their reproducibility is limited, prompting interest in genetic and molecular classifications. CD44, a transmembrane glycoprotein involved in cell adhesion and migration, has been identified as a potential marker for cancer stem cells (CSCs) in various malignancies, including EC.Objective: This study aims to evaluate the immunohistochemical expression of cluster of differentiation (CD44) in low-grade endometrial cancer and its correlation with clinicopathological characteristics.Methods: A prospective study involving 40 patients with low-grade EC diagnosed and treated at the University Clinic for Gynecology and Obstetrics in Skopje was conducted. Immunohistochemical analysis assessed CD44 expression in paraffin-embedded samples from curettage material. Tumors were staged according to FIGO classification, and various clinicopathological parameters were recorded. Results: High CD44 expression was observed in 15% of cases. The cohort's mean age was 61.5 years, with an average BMI of 32.59 kg/m^2. High CD44 expression significantly correlated with lower endometrial thickness (p=0.036). No significant associations were found between CD44 expression and and key prognostic factors such as disease stage, lymphovascular invasion, or myometrial invasion,although all cases with high CD44 expression were in stage I disease.Conclusion: Most patients with low-grade endometrial cancer exhibited low CD44 expression, suggesting its role in tumor biology and treatment strategies. Further research is needed to elucidate CD44's significance in endometrial cancer progression and its potential as a prognostic marker.

Key words: endometrial, cancer, CD44

INTRODUCTION

The most prevalent type of gynecological cancer is endometrial cancer (EC).1,2Bokhman (1983) distinguished between two clinicopathologic types of EC based on the disease's prognosis, pathologic characteristics, and endocrine and metabolic influences. These ECs are type 1, which make up roughly 70% of all ECs and are the focus of our study. They have a lower grade, primarily endometrioid histology, and are estrogen dependent. Compared to type 2 ECs, they have less myometrial invasion (5 year survival >85%). A variety of high-grade tumors with clinically aggressive histologies (serous, clear cell) make up type 2 ECs. The prognosis for these patients is worse. Despite being linked to the prognosis of endometrial cancers, histological type and

other clinicopathological parameters (FIGO stage and histologicalgrade)haveacomparativelylowreproducibility 3-5, which results in imprecise findings in clinical trials and consequently, insufficient or excessive treatment of EC patients. Over the past ten years, there has been an increase in the number of studies examining prognostic factors like histopathological type, histological grade, lymphovascular involvement and disease stage. Research on genetic carcinogenesis, such as molecular changes intended to offer a new prognostic classification, is focused on genetic carcinogenesis due to the low reproducibility of the above mentioned prognostic factors. In cases of advanced disease, the necessity of adjuvant therapy becomes more apparent. However, a number of pathologic features in patients with early-stage disease establish whether a patient requires adjuvant therapy and is at low, intermediate, or high risk of recurrence. Majority of this factors are poorly reproducible The new molecular classification offers a new reclassification according to prognostic groups, and hence de-escalation, i.e. escalation of therapy, and also opens new horizons for targeted therapy.6 In addition to the so-called classical oncology therapy in an adjuvant or neoadjuvant setting, which includes radiotherapy and chemotherapy, new drugs have also been developed among which are the immune checkpoint inhibitors, namely pembrolizumab, approved by the FDA for use in mismatch repair deficient (MMRd)/ microsatelite instable (MSI-high), while the same in combination with an angiogenesis inhibitor (Lenvatinib) is indicated in the group of endometrial cancers that are microsatellite stable (MSS)/mismatch repair profficient (MMRp). But because tumor resistance has grown, researchersare still looking for novel treatment strategies that could greatly enhance the prognosis of patients with advanced or recurrent endometrial cancer (EC). 7The theory for cancer stem cell (CSC) is one of the most intriguing strategies for overcoming drug resistance. The idea of clonal evolution, which posits that neoplasms arise through the repeated spread of pre-existing somatic mutations, has historically dominated oncology research. 8 The evidence for clonal heterogeneity of tumors leading to drug resistance is challenged by this idea. 9 The CSC theory (cancer stem cell theory), which states that tumors are composed of cells in different stages of maturation, including CSCs, is supported by a growing body of scientific data. The entire population of cellular neoplasms, including their heterogeneity, treatment resistance and invasiveness, is caused by CSCs, which are stem-like cells that have developed an oncogenic mutation and the ability to self-renew and differentiate.10.

The presence of CSCs has been confirmed in several different types of tumors including breast tumors, colorectal malignancies, prostate, central nervous system cancers, melanomas and sarcomas. During the last decades, attempts have been made to create several therapeutic strategies targeting CSCs. These include positive examples of targeted therapies for acute leukemia, sonidegib in basal cell carcinomas and imatinib targeting a signaling pathway that drives growth in gastrointestinal stromal tumors (GIST).10Different molecules have been studied as markers of origin (cancer stem cells) in endometrial cancer. Among them, cluster of differentiation 44 (CD44) represents transmembrane proteins, an adhesion molecule, which is involved in the processes of invasion and metastasis and is proposed as a marker for CSC in EC.11,12

The aim of the research is to determine the degree of immunohistochemical expression of CD44 in low-grade endometrial cancer and to examine the relationship with the clinicopathological characteristics of the disease and of the patients.

MATERIALS AND METHODS

Patients

The study was designed as a prospective, prognostic, cohort, interventional study. It included 40 patients with low-grade endometrial cancer diagnosed and operated on at the University Clinic for Gynecology and Obstetrics, Skopje in collaboration with the Institute of Pathology, Skopje, in 2023. The patients were informed and signed an informed consent to enter the study. After obtaining the histopathological diagnosis, the CD44 expression was determined immunohistochemically (IHC) on the endometrial curettage material. All patients underwent hysterectomy with bilateral salpingo-oophorectomy and sentinel pelvic lymph node biopsy. Exclusion criteria were high-grade endometrial cancer,presumed II-IV stage disease preoperatively or high ASA score. After the surgical treatment, the tumors were histopathologically analyzed at the Institute of Pathology,Skopje and staged according to the FIGO classification (FIGO 2018). Clinicopathological variables (age, body mass index index (BMI), endometrial thickness assessed by ultrasound, menopausal status, parity, presence of arterial hypertension, diabetes, FIGO stage, lymphovascular and

myometrial invasion) were obtained from the medical history and pathology report.

Immunohistochemical analysis

Microscopic diagnosis of low-grade endometrial adenocarcinoma was made on paraffin sections from delivered curettage material. After 24 hours of fixation, the samples were processed in an automatic tissue processor, and then from the same prepared in paraffin molds. 4-5 micron sections were prepared on an automatic rotary microtome and stained with basic histological staining Hemalun&Eosin. Immunohistochemical analysis was performed on the same material to determine CD44 expression. Monoclonal antibody CD44 (Phagocytic Glycoprotein-1) clone DF1485 (Agilent Technologies, CA, USA) was used for detection of expression at a dilution of 1:50.

Immunohistochemical scoring

The immunohistochemistry score (IHS) was calculated for each case individually. The percentage of positively stained epithelial cells was scored as 0-0%, 1 (1-10%), 2 (11-25%), 3 (26-50%), 4(>50%). The expression intensity was categorized into 3 groups: 1 (weak), 2 (moderate), 3 (strongly positive). We obtained the final score by multiplying the two scores, after which we made a dichotomous division of cases with low (for those with a score <10) and high score (score >10).

RESULTS

Immunohistochemical analysis for cluster of differentiation 44 (CD44), showed a high level of CD44 expression obtained by scoring in 6 cases (15%), while a low score was found in 34 (85%).

The results showed that the mean age of the study patients was 61.5 years (minimum 45 - maximum 82). Among them, the average body mass index (BMI) was 32.59 kg/m2 ±6.9 kg/m2) (19-53). Thirty-three (82.5%) of the patients were postmenopausal, while 7 patients were in the reproductive period and perimenopause (17.5%). Positive anamnestic information about abnormal vaginal bleeding was given by 36 (90%) of the respondents. A positive family history of gynecological, colorectal and breast cancer was obtained in 25% of the patients (10 patients). Parity analysis showed that most of the patients had ≤2 children (70%), while 30% (12 respondents) had >2 children. Thirty-one patients (77.5%) had arterial hypertension, including one with cerebrovascular event (2.5%) and one patient with aortic aneurysm (2.5%). 16 patients (40% of the respondents) had diabetes mellitus.

The initial diagnostic ultrasound evaluation of endometrial thickness showed that the average thickness measured in millimeters (mm) was 15.72±6.7(min 6- max 36). The stratification of the endometrial thickness by groups, in the group ≤ 20 mm and the group > 20 mm, allocated 31 patients (77.5%) and 9 (22.5 %), respectively.

The distribution by stages showed the following results: 18 (45%), 13 (32.5%), 3 (7.5%), 6 (15%) were in IA, IB, II and IIIC stage respectively. Regarding the grade, 19 (47.5%) belonged to grade 1, while 21 patients (52.5%) had grade 2 endometrial cancer. The histopathological analysis of myometrial invasion showed that myometrial invasion below 50% was recorded in 18 patients (45%), while 32 patients (55%) had myometrial invasion above 50%. Lymphovascular invasion was determined in 15 patients (37.5%). Twenty-five patients had no lymphovascular invasion (62.5%).

Patients with high CD44 expression were not significantly younger on average than patients with low expression (59.5 ± 4.2 vs 61.4 ± 9.5, p=0.63), had a non-significantly higher average BMI (35.33 ± 3.6 vs 32.09 ± 7.3 kg/m2, p=0.3) and had a significantly lower average thickness of the endometrium (10.50 ± 2.1 vs 16.65 ± 6.8 mm, p=0.036). These patients had often grade 2 endometrial cancer (66.67% vs 50%, p=0.66), more than 2 children born (50% vs 26.47%, p=0.34), were non-significantly more hypertensive than patients with low CD44 expression (83.33% vs 70.59%, p=0.45)%), insignificantly less often had bleeding (83.33% vs 91.18%, p=0.49), insignificantly less often were diabetics (16.67% vs 44.12%, p=0.064). The prevalence of myometrial invasion >50% and lymphovascular invasion was similar in the high and low CD44score groups (50% vs 55.88%,p=0.1; and, 33.33% vs 38.24%,p=1.0 respectively). The stage at which the disease was diagnosed did not differ significantly depending on the expression of CD44 (p=0.1), although in the higher stages of the disease (stage II and III) no cases with high expression were registered.

We present a table summarizing the distribution of results based on CD44 expression levels, including frequencies and levels of significance:

Table 1. This table summarizes the key findings from the study, allowing for a clear comparison between low and high CD44 expression groups across various clinical parameters.

Variabe n		CD 44 expression			p-level
		low	high		
Age (years)	mean ± SD		61.4 ± 9.5	59.5 ± 4.2	p=0.63
	min- max		45 – 82	54 – 64	
Grade	1	19	17 (50.0)	2 (33.33)	p=0.66
	2	21	17 (50.0)	4 (66.67)	
Myometrial invasion	MI < 50%	18	15 (44.12)	3 (50)	p=1.0
	MI > 50%	22	19 (55.88)	3 (50)	
Lymphpvascular invasion	yes	15	13 (38.24)	2 (33.33)	p=1.0
Stage	I A	18	15 (44.12)	3 (50)	p=0.1
	I B	13	11 (32.35)	3 (50)	
	II	3	3 (8.82)	0	
	III C1	6	5 (14.71)	0	
BMI(kg/m2)	mean ± SD		32.09 ± 7.3	35.33 ± 3.6	p=0.3
	min- max		19 – 53	30 – 40	
Endometrial thickness (mm)	mean ± SD		16.65 ± 6.8	10.50 ± 2.1	*p=0.036
	min- max		6 – 36	8 – 14	
Endometrium	≤ 20	31	25 (73.53)	6 (100)	p=0.31
	> 21	9	9 (26.47)	0	
Parity	≤ 2	28	25 (73.53)	3 (50)	p=0.34
	> 2	12	9 (26.47)	3 (50)	
Postmenopausal status	yes	33	28 (82.35)	5 (83.33)	p=1.0
Uterine bleeding	yes	36	31 (91.18)	5 (83.33)	p=0.49
Arterial hypertension	yes	29	24 (70.59)	5 (83.33)	p=0.45
	no	9	8 (23.53)	1 (16.67)	
	CVI	1	1 (2.94)	0	
	aneurism	1	1 (2.94)	0	
Diabetes melitus	yes	16	15 (44.12)	1 (16.67)	p=0.064
	no	23	18 (52.94)	5 (83.33)	
	missing	1	1 (2.94)	0	

X2(Chi-square test); t(Student t-test)

*sig p<0.05,

Notes:

- Frequencies are represented as counts (n) and percentages (%).

- p-values indicate the statistical significance of differences between low and high CD44 expression groups.

Mean values are shown with standard deviation (±) where applicable.

DISCUSSION

Endometrial cancer (EC) has emerged as a significant health concern, being the most common gynecological malignancy globally. The classification of EC into type 1 and type 2 provides a framework for understanding its biology and guiding treatment strategies. Type 1 EC, which is predominantly estrogen-dependent and characterized by low-grade histological features, is the focus of this study. The role of different markers, particularly CD44, in the prognosis and treatment of low-grade EC is gaining traction, yet it remains inadequately explored.

CD44 is a cell surface glycoprotein that serves as a receptor for hyaluronic acid and is implicated in various cellular processes, including adhesion, migration, and

signaling. Its involvement in cancer stem cell (CSC) dynamics is particularly noteworthy, as CSCs are believed to contribute to tumor heterogeneity, recurrence, and resistance to therapy. Previous studies have identified CD44 as a potential marker for CSCs in numerous cancers, including colorectal and breast cancers. In the context of EC, CD44's role remains ambiguous, with conflicting reports regarding its expression and correlation with clinical outcomes. Several studies have suggested that CD44 may have a role in EC carcinogenesis. However the results are conflicting. Most studies reveal increased CD44 expression, although the patterns differ, often yielding contradicting results.

The CD44 gene promoter is activated by the K-ras oncogene product, which alters CD44 mRNA splicing. 13,14 Although an activating mutation in codons 12 or 13 of the K-ras proto-oncogene is not present in normal endometrium, it has been identified in 10–40% of endometrial carcinomas and 6–15% of atypical hyperplasia. According to these results, CD44 has an impact on early-stage EC oncogenesis, particularly in endometrial carcinomas that have undergone significant differentiation (well differentiated EC). We can therefore conclude that the CSC-related protein CD44 may play a role in the carcinogenesis of early-stage endometrial cancers and that its overexpression may help detect endometrial cancers early. According to published research, increased CD44 expression may be a poor prognostic indicator for EC (associated with deeper myometrial invasion, lymphovascular invasion, advanced stage and higher grade). 15 Other reports found no association between CD44 and disease stage, myometrial invasion, or LVSI. 16 In the Stokes study,17 the absence of myometrial invasion was found to correlate significantly with CD44v6 expression. This could be a signal for malignancies with myometrial involvement, making it easier to qualify for lymphadenectomy before surgery. In contrast, Leblanc et al.18 discovered that as the depth of myometrial invasion increased, CD44 expression also increased. Varied methodologies and limited patient groups could aid to these inconsistent results. According to our research, only 15% of low-grade endometrial cancer cases had high CD44 expression. This implies that there is low CD44 expression in most of these tumors, which could indicate a reduced number of cancer stem-like cells. To understand the potential prognostic value of CD44, an understanding of the relationship between its expression and tumor characteristics is required. According to previous research, advanced disease features such as deeper myometrial invasion and lymphovascular involvement may be associated with high CD44 expression. However, our results did not demonstrate significant associations between CD44 expression and these key prognostic factors.Our analyzes showed that all cases of high expression were registered among patients with stage I disease. Although not significant, which may be due to the small sample, we did not register a case with high expression in the higher stages of the disease. This may indicate the hypothesized theory that CD44 expression declines with increasing tumor aggressiveness and invasion and metastasis processes, although the non-significance may be due to the small case series as mentioned above.

The predominance of low CD44 expression in our cohort raises important questions about the underlying biology of low-grade endometrial cancer. It may suggest that these tumors are less reliant on the mechanisms associated with CSCs for growth and invasion. Instead, they might be driven by other pathways or factors that are not yet fully understood. This finding aligns with the observation that low-grade tumors generally have a more indolent course and a better prognosis compared to high-grade tumors. Furthermore, the significant correlation between high CD44 expression and lower endometrial thickness is may indicate that in the early stages of tumor development, before myometrial and lymphovascular invasion occur. CD44 expression is downregulated as the tumor begins to invade the surrounding tissue. This could suggest a potential shift in the tumor's microenvironment, influencing how cancer cells interact with their surroundings. Understanding these dynamics is crucial for developing targeted therapies and intervention strategies that can effectively address the unique characteristics of low-grade EC. Similar results have been obtained in other published studies.19

Despite the potential of CD44 as a prognostic marker, our study highlights the challenges associated with its clinical utility. The lack of significant associations with other established prognostic factors—such as FIGO stage, lymphovascular invasion, and myometrial invasion—suggests that CD44 expression alone may not be sufficient to inform treatment decisions. Moreover, the heterogeneity observed in CD44 expression patterns across different studies further complicates its role in endometrial cancer prognosis.

These inconsistencies could be attributed to variations in methodologies, patient populations, and tumor biology.

Future research should aim to standardize protocols for assessing CD44 expression and explore its interactions with other molecular markers. Such comprehensive analyses could elucidate the pathways through which CD44 contributes to endometrial cancer progression and resistance.

CONCLUSION

The findings of this study indicate that the majority of patients with low-grade endometrial cancer exhibit low CD44 expression, suggesting that CD44 may play a limited role in the tumor's aggressive behavior in this subtype. Despite the known involvement of CD44 in cancer stem cell dynamics and its implications for tumor invasiveness, our results did not show significant correlations between CD44 expression and key prognostic factors such as disease stage, lymphovascular invasion, or myometrial invasion.

However, the observed association between high CD44 expression and lower endometrial thickness could indicate a specific context in which CD44 might influence early-stage tumorbehavior. Of particular importance is the observation that all cases with high expression of CD44 are in stage I of the disease.These results highlight the complexity of EC biology and the need for further research to better understand the mechanisms by which CD44 and other markers may impact prognosis and treatment decisions. Ultimately, a deeper investigation into the role of CD44 in endometrial cancer could pave the way for more tailored therapeutic approaches, particularly in distinguishing between different risk profiles and guiding treatment strategies for patients with early-stage disease.

Conflict of Interest: None declared

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