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BETA-CATENIN – AN IMPORTANT IMMUNO-HISTOCHEMICAL TOOL IN STRATIFYING ENDOMETRIAL CARCINOMAS?

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Abstract

Introduction. Recent research has demonstrated that the immunohistochemical nuclear β-catenin expression is a valid surrogate for CTNNB1 exon 3 mutation in endometrial carcinomas (ECs). This mutation is an independent prognostic factor which identifies a subgroup of low-grade endometrial carcinomas that have a tendency for recurrence and worse prognosis.
The objective of the study was to evaluate nuclear β-catenin expression in different molecular subgroups of ECs.
Material and methods. We tested immunohistochemical nuclear β-catenin expression in 50 cases of endometrial carcinomas diagnosed in two clinical institutions. Statistical analysis was performed between β-catenin expression and various clinical, demographic, pathological and immunohistochemical parameters (age, myometrial invasion, FIGO grade, histopathological subtype, hormone receptors – ER, PR etc). Additionally, we analysed what molecular subgroup

Résumé

La bêta-caténine – un outil immunohistochimique important dans la stratification des carcinomes endométriaux?

Introduction. Des recherches récentes ont montré que l'expression de la β-caténine nucléaire immunohistochimique est un substitut valide pour la mutation de l'exon 3 CTNBB1 dans les carcinomes de l'endomètre (CE). Cette mutation est un facteur pronostique indépendant et identifie un sous-groupe de carcinomes de l'endomètre à bas degré qui ont une tendance à la récidive et à un pronostic défavorable.
L'objectif de l'étude était d'évaluer l'expression de la β-caténine nucléaire dans différents sous-groupes moléculaires de CE.
Matériel et méthodes. Nous avons testé l'expression immunohistochimique de la β-caténine nucléaire dans 50 cas de carcinomes de l'endomètre diagnostiqués dans deux unités cliniques. Une analyse statistique a

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of ECs (MSS, MSI, p53wt, p53abn) revealed the most frequent cases with β-catenin expression.
Results. Our study indicated that ECs with nuclear β-catenin positivity were observed in cases with higher FIGO grade (p=0.02), in endometrioid carcinomas (p=0.04) and in cases with lympho-vascular invasion (p=0.05). ER and PR were frequently expressed in the positive β-catenin subgroup (p=0.03, p=0.02). Our results show that ECs which express nuclear β-catenin correlate with parameters that are already considered unfavourable.
Conclusions. mmunohistochemical β-catenin nuclear expression is an excellent replacement for the CTNNB1 exon 3 mutation in ECs and helps to stratify and predict prognosis in certain cases of ECs. We believe that future research will include this marker as part of the routine immunohistochemical panel for ECs.

Keywords: endometrial carcinoma, β-catenin, prognosis.

Abbreviations:
CNH – Copy number high
CNL – Copy number low
EC – Endometrial Carcinoma
ER – Estrogen Receptor
ESMO – European Society for Medical Oncology
FIGO – Fédération Internationale de Gynécologie et d`Obstétrique
LVSI – Lympho-vascular invasion
MSI-H – Microsatellite instability – hypermutated
MSS – Microsatellite Stable
NSMP – Non-specific molecular profile
PD-L1 – Programmed Death – Ligand 1
POLE – Polymerase-epsilon
TCGA – The Cancer Genome Atlas
TILs – Tumour Infiltrating Lymphocytes
TMA – Tissue Microarray

été réalisée entre l'expression de la β-caténine et divers paramètres cliniques, démographiques, pathologiques et immunohistochimiques (âge, invasion myométriale, grade FIGO, sous-type histopathologique, récepteurs hormonaux – ER, PR, etc.). De plus, nous avons analysé lequel sous-groupe moléculaire de CE (MSS, MSI, p53wt, p53abn) présentait les cas les plus fréquents d'expression de la β-caténine.
Résultats. Notre étude a révélé que des CE avec une positivité à la β-caténine nucléaire étaient observées dans les cas de grade FIGO plus élevé (p = 0,02), dans les carcinomes endométrioïdes (p = 0,04) et dans les cas d'invasion lympho-vasculaire (p = 0,05). ER et PR étaient fréquemment exprimés dans le sous-groupe de β-caténine positive (p = 0,03, p = 0,02). Nos résultats montrent que les CE qui expriment la β-caténine nucléaire sont en corrélation avec des paramètres déjà considérés comme défavorables.
Conclusions. L'expression nucléaire immunohistochimique de la β-caténine est un excellent remplacement de la mutation de l'exon 3 CTNBB1 dans les CE et aide à stratifier et à prédire le pronostic dans certains cas de CE. Nous pensons que les recherches futures incluront ce marqueur dans le cadre du panneau immunohistochimique de routine pour les CE.

Mots-clés: carcinome de l'endomètre, β-caténine, pronostic.

Introduction

Endometrial carcinoma (EC) is the sixth most frequent neoplasia in women[1]. In 2013, The Cancer Genome Atlas (TCGA) published a new molecular classification based on clinical prognosis: Polymerase-epsilon (POLE) ultra-mutated, microsatellite instability hypermutated, copy number low (CNL) and copy-number high (CNH)[2]. The CNL molecular subgroup is very heterogenous regarding survival and clinical behaviour, so there have been numerous studies that looked for prognostic markers to further stratify this category[3].

CTNNB1 exon 3 mutation has been identified as an independent prognostic marker for low-grade ECs and has been associated with higher rates of recurrence and worse survival[3]. This gene encodes the protein β-catenin which is involved in the WNT-pathway[4] and for that matter, we can use immunohistochemical analysis to assess nuclear β-catenin positivity. Researchers have found that there is a very good correlation between the CTNNB1 mutation and the immunohistochemical expression of nuclear β-catenin positivity in ECs[3,5,6].

The objective of the study was to evaluate nuclear β-catenin positivity in ECs in a group of Romanian patients and assess whether this immunohistochemical marker has prognostic value and can be used to predict a worse outcome.

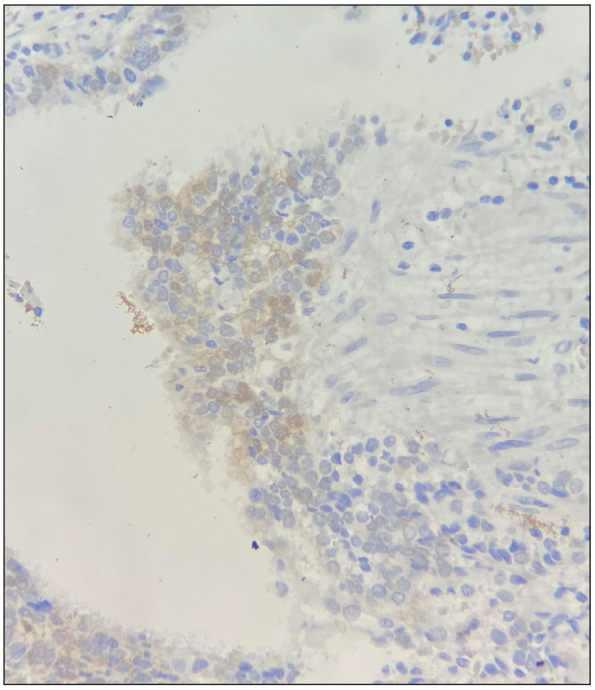

Figure 1. Nuclear expression of β-catenin in endometrial carcinomas (400x).

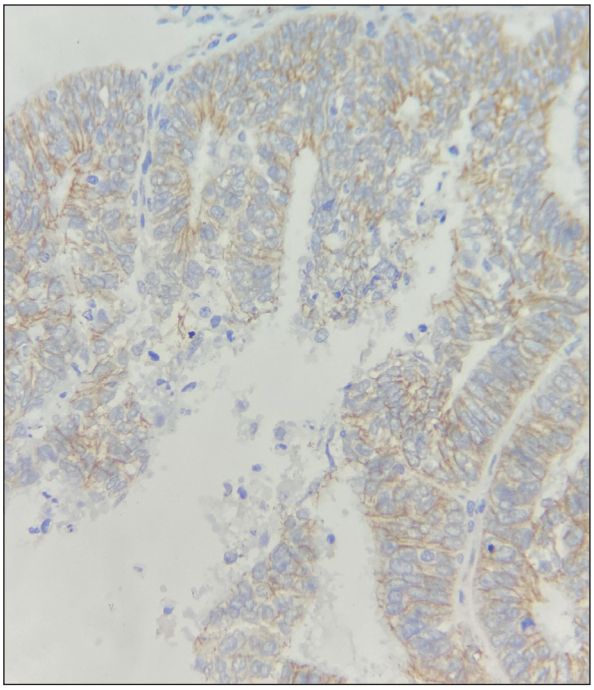

Figure 2. Membranous expression of β-catenin in endometrial carcinomas as internal control (200x).

Materials and methods

This study obtained approval from the Ethics Committees of two different institutions in Bucharest, Romania: The Emergency University Hospital and "Sf. Maria" Clinical Hospital. Informed consents were signed by all patients. The cases selection was performed from a previous database[7] and included 50 cases of ECs from the Pathology Department archives, diagnosed between 2014 and 2019. Molecular classification for ECs was already performed and divided the cohort into four subgroups: MSS subgroup, MSI subgroup, p53abn subgroup and p53wt subgroup[7]. In addition, Programmed Death – Ligand 1 (PD-L1) testing was assessed on this cohort[8].

The obtained samples of endometrial carcinoma were reviewed, cored (1mm) in triplicate and arrayed as previously described[7]. Tissue sections were stained with antibodies against β-catenin (Ventana, catalogue number 760-4242, clone 14, Mouse) and protocol was followed for staining. Immunohistochemistry for β-catenin was performed on all cases (n=50) and any definitive nuclear staining for β-catenin was interpreted as positive (Figure 1). Membrane staining for β-catenin was considered positive internal control, but without nuclear staining it was not included in our study (Figure 2).

The statistical analysis used IBM SPSS Statistics, version 26. Independent t test was used for analysis of variance for continuous variables (example: age), chi-square test and Fisher's exact test for correlations of demographic, clinical and pathological data, immunohistochemical staining pattern and β-catenin subgroups. All tests were two-sided and statistical significance was defined as a p-value <0.05.

Results and discussion

Clinical and demographic descriptive statistics (Table 1) exhibited a higher mean age for diagnosis in endometrial carcinomas with positive nuclear β-catenin (65.55 years) than the negative subgroup, with patients ranging from 39 years to 86 years. This finding is unusual, as other studies reported this molecular alteration in younger women[3]. Only one patient with omentectomy with tumour involvement showed nuclear positivity for β-catenin, but there was no statistical significance associated. Nonetheless, even low-grade ECs with β-catenin mutation may behave aggressively and disseminate into the peritoneum[9]. A tumour size > 2cm was noticed in 8/11 cases of ECs with β-catenin positivity. Although there were no statistical differences, this feature outlines a subgroup of ECs with a worse prognosis and clinical behaviour[10-12]. Endometrial premalignant lesions were identified in the β-catenin positive subgroup, which is not surprising considering the studies expressing that this immunohistochemical marker can be used as a tool to predict malignancy[13].

The histopathological subtyping revealed the fact that all 11 cases (22%) of ECs positive for β-catenin

Table 1. Correlations between β-catenin expression and clinical, pathological and immunohistochemical parameters in endometrial carcinomas (n=50).

	β-catenin positive (nuclear) n=11	*β-catenin negative (nuclear) n=39*	p
Age (y)			
mean	65.55	62.31	p=0.16 ˢ
Omentectomy			
positive	1 (2%)	6 (12%)	p=0.7 ≈
negative	2 (4%)	4 (8%)	
absent	8 (16%)	29 (58%)	
Peritoneal cytology			
positive	0 (0%)	3 (6%)	p=0.4 ≈
negative	2 (4%)	3 (6%)	
absent	9 (18%)	33 (66%)	
Cervical cytology			
positive	0 (0%)	4 (8%)	p=0.4 ≈
negative	1 (2%)	5 (10%)	
absent	10 (20%)	30 (60%)	
Tumour size			
≤2 cm	3 (6%)	14 (28%)	p=0,5 ≈
>2 cm	8 (16%)	25 (50%)	
Preneoplastic lesions			
Endometrial hyperplasia without atypia	1 (2%)	0 (0%)	p=0,1 ≈
Endometrial hyperplasia with atypia/EIN	1(2%)	3 (6%)	
Absent	9 (18%)	36 (72%)	
Histopathological subtype			
Endometrioid	11 (22%)	30 (60%)	**p=0.04** ≈
Serous	0 (0%)	4 (8%)	
Clear cell	0 (0%)	2 (4%)	
Mixed	0 (0%)	2 (4%)	
Carcinosarcoma	0 (0%)	1 (2%)	
FIGO Grade			
Grade 1	1 (2%)	8 (16%)	**p=0.02** ≈
Grade 2	1 (2%)	14 (28%)	
Grade 3	3 (6%)	17 (34%)	
pT	1 (2%)	8 (16%)	p=0.6 ≈
T1	2 (4%)	11 (22%)	
T2	3 (6%)	8 (16%)	
T3a	4 (8%)	7 (14%)	
T3b	1 (2%)	5 (10%)	
pN			
N0	8 (16%)	24 (48%)	p=0.5 ≈
N1	0 (0%)	6 (12%)	
N2	0 (0%)	1 (2%)	
absent	3 (16%)	8 (16%)	
pM			
M0	11 (70%)	35 (70%)	p=0.5 ¯
M1	0 (0%)	4 (8%)	
FIGO stage			
IA	1 (2%)	8 (16%)	p=0.2 ≈
IB	2 (4%)	10 (20%)	
II	3 (6%)	7 (14%	
IIIA	4 (8%)	3 (6%)	
IIIB	1 (2%)	4 (8%)	
IIIC1	0 (0%)	3 (6%)	
IIIC2	0 (0%)	4 (8%)	
Tumour necrosis			
present	9 (18%)	30 (60%)	p=0.7 ≈
absent	2 (4%)	9 (18%)	

	β-catenin positive (nuclear) n=11	*β-catenin negative (nuclear) n=39*	p
Adenomyosis			
present, positive	0 (0%)	2 (4%)	p=0.7 ≈
present, negative	4 (8%)	12 (24%)	
absent	7 (14%)	25 (50%)	
LVSI			
present	6 (12%)	31 (62%)	**p=0.05** ≈
absent	5 (10%)	8 (16%)	
TILs			
low	2 (4%)	12 (24%)	p=0.7 ≈
moderate	7 (14%)	21 (42%)	
marked	2 (4%)	6 (12%)	
Myometrial invasion			
<50%	5 (10%)	14 (28%)	p=0.7 ≈
≥50%	6 (12%)	25 (50%)	
Pattern of invasion			
Pushing	5 (10%)	11 (22%)	p=0.1 ≈
Diffusely infiltrative	5 (10%)	26 (52%)	
Adenomyosis involvement	0 (0%)	2 (4%)	
MELF	1 (2%)	0 (0%)	
ESMO criteria			
Low Risk	1 (2%)	2 (4%)	p=0.1 ≈
Intermediate Risk	0 (0%)	4 (8%)	
High Intermediate Risk	2 (4%)	13 (26%)	
High	6 (12%)	8 (16%)	
Advanced	2 (4%)	12 (24%)	
ER			
positive	11 (22%)	32 (645)	**p=0.03** ¬
negative	0 (0%)	7 (14%)	
PR			
positive	11 (22%)	31 (62%)	**p=0.02** ¬
negative	0 (0%)	8 (16%)	
HER2			
0/+1	11 (22%)	38 (76%)	p=1 ≈
3+	0 (0%)	1 (2%)	
P53			
P53abn – overexpression	1 (2%)	5 (10%)	p=0.7 ≈
P53abn – null	1 (2%)	6 (12%)	
P53wt	9 (18%)	28 (56%)	
MSS	8 (16%)	26 (52%)	
MSI	3 (6%)	13 (26%)	p=1 ¬
PD-L1			
<1%	8 (16%)	22 (44%)	p=0.4 ≈
1-49%	3 (6%)	14 (28%)	
≥50%	0 (0%)	3 (6%)	

ς : independent t test;≈ : chi-square test; ¬ : Fisher's exact test.
Legend: EIN – Endometrial intraepithelial neoplasia; LVSI – lympho-vascular invasion; TILs – tumour infiltrating lymphocytes.

were endometrioid, showing statistical significance (p=0.04). These results complete other studies that prove this subtype is usually characterized by an abnormality in the Wnt/β-catenin pathway, and therefore by nuclear β-catenin positivity[6, 14-16].

Three cases (6%) of ECs with nuclear positivity for β-catenin expressed statistical significance with high-grade tumours – FIGO grade 3 (p=0.02). Most studies indicate that the Wnt/ β-catenin pathway is present in almost all cases in endometrioid low-grade carcinomas[3,6] and very few researchers have reported β-catenin nuclear positivity in high-grade non-endometrioid carcinomas[16]. TNM staging revealed that most cases were found in the T3a subcategory, outlining the poor prognosis of the positive β-catenin subgroup. On the other hand, most cases were observed in the N0 subcategory and M0 subcategory, outlining the low propensity of these cases for lymph

node metastasis and distant metastasis, as in other research[3,17,18]. Positive β-catenin subgroup showed that the most frequent FIGO stage was IIIA, with variable distribution among FIGO stage IA, IB and II. Reportedly, endometrioid carcinomas which express β-catenin usually present lower FIGO stages[19].

Tumour necrosis was more frequent in the positive subgroup (18% of cases) and moderate tumour infiltrating lymphocytes (TILs) were noticed in 14% of cases. Most patients with concomitant adenomyosis expressed β-catenin in 14% of cases, while 8% of them expressed β-catenin in cases with adenomyosis with tumour involvement. There are studies that have found a clear link between mutations in the Wnt/β-catenin pathway and the development of adenomyosis using epithelial-mesenchymal transition[20-22]. Lympho-vascular invasion (LVSI) was statistically correlated with positive β-catenin subgroups (p=0.05). This finding is not unusual, as LVSI is an independent prognostic marker and is associated with worse recurrence-free survival in a similar manner with the CTNNB1- mutated ECs[3,23]. Positive β-catenin ECs showed a variable distribution of cases regarding myometrial invasion (<50% with 5 cases and ≥ 50% with 6 cases, p=0.7). Other studies did not find statistical significance for this parameter[3,6].

Nuclear β-catenin positivity was identified in ECs with pushing and diffusely infiltrative pattern of invasion in a variable manner, while the MELF pattern was present only in this category, outlining the poor outcome of this subgroup[23]. European Society for Medical Oncology (ESMO) criteria place this subgroup in the high risk category (12%)[24] .

Hormone receptors (ER and PR) presented statistical correlations with β-catenin subgroups (p=0.03, p=0.02). All positive β-catenin ECs were ER positive and PR positive, which is not unusual since positive β-catenin cases are usually endometrioid and express hormone receptors[6,17,23]. HER2 was positive in a β-catenin negative EC, serous subtype, highlighting that certain histological subtypes do not present the CTNNB1 mutation[25].

Regarding molecular subgroups of ECs, most positive cases (16%) were found in the microsatellite-stable subgroup (MSS) and the p53wt (18%) subgroup. Our data are similar with other research[3,6], confirming that nuclear positivity for β-catenin is a usual finding in the CNL or NSMP subgroups, according to TCGA[2]. Furthermore, we tested for PD-L1 and we found only three cases that showed 1-49% positivity in the β-catenin positive subgroup, with no statistical significance. Numerous clinical trials have tested immunotherapy in endometrial carcinomas, metastatic or recurrent, and have found promising results for certain molecular subgroups such as POLE-mutated ECs and MSI-H ECs [26-28]. There are only a few studies in literature that have investigated PD-L1 expression in low-grade ECs with CTNNB1 mutation. Therefore, immunotherapy has not been included as a treatment option for this category and new research is needed.

The analysis of overall survival did not reveal any statistical significance between the β-catenin positive subgroup and β-catenin negative subgroup (p=0.8). However, we noticed an 81.8% overall survival in the β-catenin positive subgroup, slightly lower than the 84.6% overall survival in the β-catenin negative subgroup.

These results demonstrate that ECs with nuclear β-catenin positivity statistically corelate with unfavourable clinical, pathological and immunohistochemical data: FIGO grade, histopathological subtype, lympho-vascular invasion. ER and PR receptors were found in the β-catenin positive subgroup, which usually points towards a favourable clinical behaviour. This study has a sample size limitation; however, despite the heterogeneity in NSMP subgroup, β-catenin could represent an important tool for prognosis.

Conclusions

In an era of evolving cancer classifications with access to genetic mutation testing, our struggle remains to find affordable, reliable and easy to use prognostic markers in our routine practice. β-catenin is an affordable immunohistochemical marker for most laboratories and represents thus far a very good prognostic marker, especially for predicting recurrence in low-grade endometrioid carcinomas.

Authors' contributions

AE wrote the manuscript. AE and AB performed immunohistochemistry and made substantial contributions to analysis and interpretation of data. AE and MG conceived and designed the study and NC and MS gave final approval of the version to be published. All authors read and approved the final manuscript.

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Availability of data and materials: *The datasets used and/or analysed during the present study are available from the corresponding author on reasonable request.*

Ethics approval and consent to participate:

The study was approved by the Ethics Committees of the Emergency Universitary Hospital and "Sf. Maria"

Clinical Hospital, Bucharest, Romania (31673/1.07.2020). Signed written informed consents were obtained from all the patients.

Competing interests: *The authors declare that they have no competing interests.*

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