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THE PROGNOSTIC ROLE OF BETA-CATENIN IN PATIENTS WITH ADVANCED STAGE SEROUS OVARIAN CANCER

Β-КАТЕНИНОТ И НЕГОВАТА ПРОГНОСТИЧКА УЛОГА КАЈ ПАЦИЕНТКИ СО НАПРЕДНАТ СТАДИУМ НА СЕРОЗЕН ОВАРИЈАЛЕН КАРЦИНОМ

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Abstract

Introduction. Serous ovarian cancer is the most common sub-type of epithelial ovarian cancer and is the leading cause of cancer-related death among gynecologic cancer patients. Beta-catenin plays a vital role in the genesis of certain types of cancers. Its implications in the survival and prognosis of patients with serous ovarian cancer is not yet fully understood.
The aim of the study was to analyze the association between beta-catenin expression, as well as certain other clinical and pathohistological characteristics of serous ovarian cancers, with the overall patient survival in advanced stage cases.
Methods. We conducted immunohistochemical analysis in tumor specimens from 40 patients to determine the expression of beta-catenin. We analyzed the relationship between beta-catenin expression and the FIGO disease stage and the tumor grade. We used Kaplan-Meier statistics to analyze the prognosis.
Results. We detected increased expression of beta-catenin in patients with FIGO Stage III or IV (p= 0.0003). We did not detect a statistically significant association between beta-catenin expression and tumor grade (p=0.817). The positive expression of beta-catenin was associated with shorter average survival (p= 0.034). There was no statistically significant relationship between beta-catenin expression and other pathohistological tumor features.
Conclusion. Beta-catenin expression is associated with poorer prognosis in patients with serous ovarian cancer.

Keywords: ovarian cancer, beta-catenin

Апстракт

Вовед. Оваријалниот серозен крцином претставува најчест поттип од епителните оваријални карциноми и претставува водечка причина за смртен исход во групата на гинеколошки карциноми. Се наметнува неопходна потреба за развивање дијагностички и прогностички маркери за ова заболување. β-катенинот игра централна улога во туморогенезата на одредени типови карциноми. Нецелосно е разјаснета улогата на β-катенинот во дијагнозата и во прогнозата на оваријалниот серозен карцином.
Цел. Целта на оваа студија е да ја анализира корелацијата меѓу експресијата на β-катенинот и клиничките и патохистолошките карактеристики, кај серозен оваријален карцином во напреднат стадиум и неговата корелација, во однос на севкупното преживување.
Методи. Во оваа студија, експресијата на β-катенинот беше испитана кај 40 пациентки со оваријален серозен карцином преку спроведени имунохистохемиски анализи. Анализирани беа соодносот меѓу експресијата на β-катенинот и FIGO стадиумот на болеста и патолошкиот градус. Со помош на Kaplan–Meier методата е направена анализа на прогнозата.
Резултати. Зголемената експресија на β-катенинот беше детектирана кај пациентки со FIGO стадиум III и IV (p=0.003). Не е утврден сигнификантен сооднос меѓу експресијата на β-катенинот и патолошкиот градус (p=0.817). Позитивната експресија на β-катенинот е поврзана со пониска стапка на преживување (p=0.034). Не е утврдена статистичка сигнификантност меѓу експресијата на β-катенинот и другите патолошки параметри.
Заклучок. Експресијата на β-катенинот може да користи како предиктивен маркер за лоша прогноза кај пацинтки со напреднат стадиум на серозен оваријален карцином.

Клучни зборови: оваријален карцином, бета-катенин

Introduction

Ovarian cancer is the fifth most common gynecologic

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malignancy in the developed world and the leading cause of cancer-related death in women with gynecologic malignancies. It accounts for 1.3% of all cancers in the USA. The National Cancer Institute approximates that in 2017, 22440 women in the US would be diagnosed with ovarian cancer, and 14080 of patients would die as a result of the disease [1]. The high incidence and mortality could be partially explained by the fact that most patients are diagnosed in advanced stage because of the insidious nature of the disease and the vague early symptoms. There have been advances in the past couple of decades in the treatment of the disease, including surgical technique advances, as well as the development of new generations of chemotherapy, but in spite of that, the overall five-year survival rates of advanced stage patients have remained relatively constant at around 28.8% [1]. Most of these patients experience recurrences and chemo-resistance. The most important tumor-associated prognostic factors for survival are disease stage, residual tumor size, histologic subtype of the tumor and the tumor grade. It is therefore prudent to investigate specific prognostic markers for ovarian cancer survival. The unraveling of the molecular mechanisms behind the pathogenesis of ovarian cancer could lead to improvement in the treatment modalities which in turn could improve survival.

Beta-catenin is a multi-functional cytoplasmic protein. Its gene is located on the 3p21 chromosome. Beta-catenin, together with E-cadherin plays a vital role in the forming of the cyto-skeleton. Downregulation of beta-catenin expression in the cell membrane has been noted in advanced stages of serous and clear cell ovarian cancers, which is associated with poor tumor differentiation and the presence of metastasis [2]. Not only does beta-catenin serve as the "inter-cellular glue" in complex with the E-cadherin, but also plays a pivotal role in the tumor genesis **[3].** The Wnt protein, which acts on the cell membrane via frizzled and lipoprotein receptor-related proteins, inhibits the phosphorylation and the degradation of beta-catenin [4].

It is noteworthy that there is only limited amount of data that regards the prognostic value of the immune expression of beta-catenin in serous ovarian cancer and the association of beta-catenin expression with the clinical and pathological characteristics of the tumor and the overall survival rate. In this study we will detect beta-catenin expression in a set of samples from serous ovarian cancer.

Materials and methods

Patients

We analyzed data obtained from the medical records of all patients hospitalized for surgical treatment of advanced stage serous ovarian cancer at the University Clinic of Gynecology and Obstetrics, University of "Ss. Cyril and Methodius", Skopje, Macedonia from 01/01/2010 to 31/12/2012. All patients were followed up at least 36 months. All histological analyses were done at the Institute of Pathology, University of "Ss. Cyril and Methodius", Skopje, Macedonia. We included data from 40 patients with advanced stage serous ovarian cancer (FIGO stages III and IV).

We recorded and analyzed the following parameters: age, disease stage (according to FIGO), tumor grade (classified as low or high based on the two-tier grading system recommended by Shimazu and Silverberg [5].

Immunohistochemistry

The immunohistochemical analysis of beta-catenin expression was done using a monoclonal antibody-Human β-catenin, clone 17C2 (Novocastro), diluted to 1:100.

The results from the immunohistochemical staining were analyzed on a NIKON 80 light microscope and were phot-documented. All specimens were analyzed by three independent observers, blinded for the clinical outcome.

The beta-catenin staining of the cell membrane, cytoplasm and nucleus was evaluated according to the description published by C.M. Lee *et al*. [6]. The staining of the cytoplasm and nucleus was considered positive. The immunohistochemical staining of cancer cells was done in a semi-quantitative fashion depending on the percentage of positive cells. The percentage of stained cells in each section was coded as follows: "0" - <5% cells stained, "1" 5-50% of the cells stained and "2" if over 50% of the cells were stained [6].

Statistical analysis

All statistical calculations were performed in MedCalcver 12.1.4.0 2011 statistical software (Broekstraat at 52, 9030 Mariakerke, Belgium). The statistical significance of the differences was analyzed using the Chi-square test. The univariate survival analysis was done using the Kaplan-Meier method. Overall survival was defined as the time between the date of the initial surgical treatment and the date of the last follow-up and/or the date of death (if the death was cancer-related). Values for $p < 0.05$ were considered statistically significant.

Results

The average age of the patients was 52 years (range 25-79 years). Thirty-six patients (90%) were in FIGO stage III, while 4 patients (10%) were FIGO stage IV. During the follow-up period, of the 36 patients included in the study, 25 patients died as a result of the disease. The average follow-up duration was 24 months (range 12-36 months), while the average survival was 15 months (range 7-23 months).

Seventeen patients (42.5%) had low-grade tumors, while 23 (57.5%) patients had high-grade tumors (Table 1). Beta-catenin proteins were located on the cell membrane, cytoplasm and nucleus of the ovarian cancer cells. Predominantly positive immuno-staining was observed on the cell membrane, as well as the cytoplasm (Figure 2 and 3). We did not observe isolated membrane beta-catenin positivity in the analyzed ovarian cancer cells.

Table 1. Association of beta-catenin, FIGO disease stage and tumor grade in serous ovarian cancer

Parameter	No. of patients (%) Beta-catenin			Total	P
	-	+	++		
Stage					
III	6 (16.6)	16 (44.4)	14 (38.8)	36 (90)	0,003
IV	0	1 (25)	3 (75)	4 (10)	
Tumor grade					
Low	3 (17,6)	5 (29.4)	9 (52.9)	17 (42.5)	0,817
High	3 (13)	9 (39.1)	11 (47.8)	23 (57.5)	

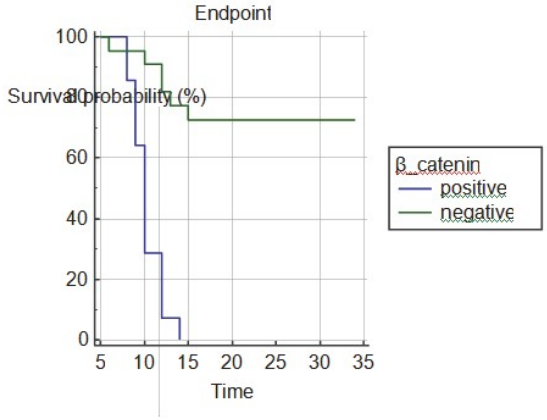

Fig. 1. Overall survival and beta-catenin expression in 40 patients with advanced stage serous ovarian cancer (Kaplan-Meier analysis, p=0.034)

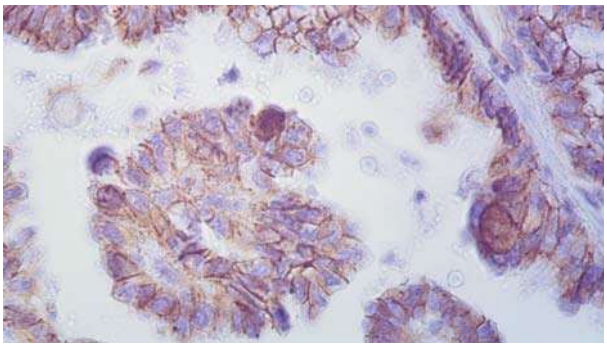

Fig. 2. Membrane and cytoplasmic expression of beta-catenin in a case of high-grade serous ovarian cancer (x400)

All stage III and IV cancers expressed a marked positivity for beta-catenin, independently of tumor grade. Indeed, 82% of the low-grade and 87% of the high-grade serous ovarian cancers manifested positive beta-catenin staining (Table 1).

During the follow-up period, 25 patients (62.5%) died as a result of causes associated with ovarian cancer. An increase of beta-catenin expression was signifycantly associated with poor overall survival (p=0.034) (Figure 1).

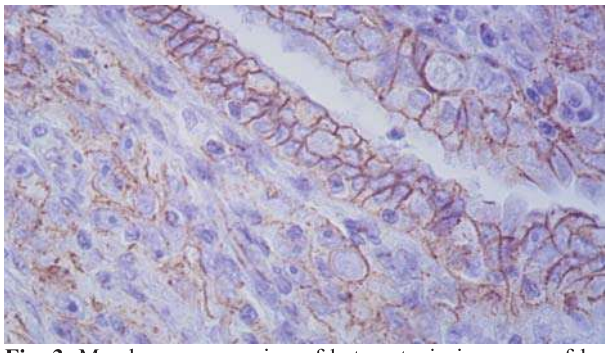

Fig. 3. Membrane expression of beta-catenin in a case of low-grade serous ovarian cancer (x400). A loss of expression is visible in a proportion of the cells

Discussion

Previous studies have associated beta-catenin with the oncogenic activity of human cancers. An increasing amount of data points towards the fact that beta-catenin is involved in the carcinogenesis, progression and metastatic spread of ovarian cancers [2, 7-10]. The role of the beta-catenin protein is different and depends on its location within the cell. E-cadherin plays an important role in the forming of cell junctions and is the key regulator of the differentiated phenotype of epithelial cells. E-cadherin forms complexes with other membrane proteins, including beta-catenin. The downregulation of membrane expression of beta-catenin is associated with poor histologic differenttiation of cancer, increased risk of local invasion and metastatic spread of the tumor, which in turn is associated with poorer survival in such patients. The so-called Wnt pathway is another molecular mechanism that includes beta-catenin and is implicated in the process of tumor genesis. The activated beta-catenin within the Wnt pathway is accumulated in the cytoplasm and nucleus. In the current study, beta-catenin was primarily located on the surface membrane and cytoplasm of the ovarian cancer cells. We only found a small number of specimens with positive nucleus staining (2 out of the 40 serous ovarian cancers). We determined that

the loss of expression of beta-catenin on the cell membrane was often associated with a high tumor grade. We focused on the relation between beta-catenin presence in the cytoplasm and nucleus and the clinical and pathologic features of the cancer. Our data showed that the expression of beta catenin was positively associated with the advanced FIGO stage of disease, but was tumor-grade independent. Beta-catenin expression was also associated with significantly poorer prognosis. Lee *et al*. published that beta-catenin expression in the nucleus was associated with a high to moderate survival rate in patients with serous ovarian cancer [6]. Their data also illustrated that the beta-catenin distribution in the cell membrane, cytoplasm or nucleus was tumor-grade independent. Our data did not confirm that beta-catenin was exclusively present in the nucleus or the cell membrane of ovarian cancer cells.

In conclusion, increased expression of beta-catenin is detected more often in patients with advanced stage serous ovarian cancer. We did not find a significant association between beta-catenin expression and tumor grade. Positive beta-catenin staining points towards poorer survival rates. Therefore, beta-catenin could be used as a marker to determine the sub-group of serous ovarian cancer patients with increased risk of poor clinical outcomes.

Conflict of interest statement. None declared.

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