Expression of podoplanin in ovarian clear cell carcinoma

Ekspresja podoplaniny w raku jasnokomórkowym jajnika

Kotarski Jozef[1], Marzec-Kotarska Barbara[2], Szumiło Justyna[3], Klepacz Robert[3], Cybulski Marek[4], Małgorzata Sobstyl[5], Bednarek Wiesława[1]

[1] 1st Dept of Gynecological Oncology and Gynecology, Medical University of Lublin, Poland
[2] Dept. of Clinical Genetics, Medical University of Lublin, Poland
[3] Dept. of Clinical Pathomorphology, Medical University of Lublin, Poland
[4] Dept. of Biochemistry and Molecular Biology, Medical University of Lublin, Poland
[5] Dept. of Gynecology and Gynecological Endocrynology, Medical University of Lublin, Poland

Abstract

Introduction: *Podoplanin is a transmembrane glycoprotein expressed in endothelial lymphatic cells. It was proven to be a predictive marker in a variety of cancers e.g. mesothelioma and head and neck squamous-cell carcinoma. Ovarian clear cell carcinoma (OCCC) is a rare and unique histopathologic subtype of epithelial ovarian cancer (EOC). The molecular basis of that phenomenon remains unknown.*

Objectives: *The aim of our study was to assess podoplanin expression on the protein level in OCCC.*

Material and Methods: *Immunohistochemistry was performed on paraffin-embedded tissues from 19 patients with diagnosed OCCC.*

Results: *Podoplanin expression was present (moderate or strong) in 52% of OCCC cases (10/19). Nine of eleven (81,2%) postmenopausal and one of eight (12,5%) premenopausal women were podoplanin positive. No differences in podoplanin expression were found in relation to clinical features of the tumor.*

Conclusion: *The incidence of podoplanin expression is higher in ovarian clear cell adenocarcinoma in postmenopausal patients.*

Key words: **clear cell carcinoma** / **ovarian epithelial cancer** / **TMA** /

Corresponding author:
Wiesława Bednarek
1st Department of Oncological Gynecology and Gyecology, Medical University of Lublin, Poland
Poland, 20-081 Lublin, Staszica 16
tel./fax: +48 81 53 27 847
e-mail: wbed@wp.pl

Otrzymano: **25.02.2011**
Zaakceptowano do druku: **29.04.2011**

Streszczenie

Wstęp: *Podoplanina jest przezbłonową glikoproteiną występującą w komórkach śródbłonka naczyń limfatycznych. Swoje zastosowanie jako marker predykcyjny znalazła w diagnostyce międzybłoniaka czy też w raku głowy i szyi. Jasnokomórkowy rak jajnika (OCCC) jest rzadko występującym i odmiennym histopatologicznym podtypem nabłonkowego raka jajnika. Molekularne podłoże tego zjawiska nadal nie jest znane.*

Cel: *Celem badań była ocena ekspresji podoplaniny na poziomie białka w jasnokomórkowym raku jajnika.*

Materiał i metody: *Ekspresję podoplaniny oceniono metodą immunohistochemiczną z zastosowaniem techniki macierzy tkankowych (TMA) u 19 pacjentek z OCCC.*

Wyniki: *Ekspresja podoplaniny była obecna (średnia lub wysoka) w 52% przypadków OCCC (10/19). Jej ekspresję wykazano u jednej spośród ośmiu pacjentek przed menopauzą (12,5%) i u 9 spośród 11 (81,2%) po menopauzie. Nie wykryto różnic w ekspresji podoplaniny w odniesieniu do cech klinicznych nowotworu.*

Wnioski: *Częsta ekspresja podoplaniny jest charakterystyczna dla kobiet po menopauzie ze zdiagnozowanym rakiem jasnokomórkowym jajnika.*

Słowa kluczowe: **rak jasnokomórkowy** / **nabłonkowy rak jajnika** / **TMA** /

Introduction

Podoplanin is a 38 kDa transmembrane glycoprotein, specifically expressed in lymphatic endothelial cells and for that reason it has been recognized as a marker for lymphangiogenesis [1, 2]. The role of podoplanin in the process of metastasis has not been conclusively determined. Wicky et al., in their tests *in vitro* and *in vivo* proved that increased expression of podoplanin leads to the decrease in adhesion and stimulation of pathologic cell migration of pectoral gland tumor, even in the presence of E-cadherin [3]. Podoplanin possesses a platelet aggregation-stimulating *(*PLAG*)* domain which takes part in blood platelets activation and aggregation. Kunita et al., in their research on animal models revealed that it visibly increases the ability of cancer cells in the circulating blood to metastasize. The ability has been significantly compromised after introducing mutations in the DNA coding the PLAG domain [4]. Therefore, podoplanin has been claimed to play the role of a certain 'intensifier', and in some cases a modulator, of tumor progression [1]. Ozaki et al., claim that podoplanin may induce platelet aggregation also by interacting with its CLEC-2 receptor (C-type lectin-like receptor 2) [5]. One of the mechanisms which explain the phenomenon of cancer cells hiding from the immune system may be the expression of podoplanin which, binding with the CLEC-2 receptor, activates blood platelets and creates a thrombus around the tumor [6].

Clear cell carcinoma accounts for under 5% of all ovarian tumors [7]. Partial response to therapy is the most characteristic feature which distinguishes clear cell carcinoma from the rest of ovarian tumors [8, 9]. Literature offers the reports by Oe et al., who assessed the expression of podoplanin in clear cell carcinoma [10] and found it to be significantly increased in OCCC, in comparison with other histologic types of ovarian cancer.

The aim of our work was to examine immunohistochemical expression of podoplanin in ovarian clear cell carcinoma.

Material and methods

19 patients with clear cell carcinoma, who had undergone surgery at I Clinic of Oncological Gynecology and Gynecology, Medical University of Lublin, were included into the study. The study has been approved by Bioethics Committee, Medical University of Lublin. The tests were performed by using tissue microarray technique (TMA). Cylindrical tissue array blocks were cut out of paraffin-embedded tissue block and immersed in the recipient blocks, especially designed for the purpose of the study, with the use of the TMA Olympus device. Representative cancerous tissue specimens, 2mm in diameter, were cut on the basis of hematoxylin and eosin staining. Immunohistochemical staining was performed according to the procedure published by Lesiak et al. [11]. The expression of podoplanin with the use of rabbit polyclonal antibody at 1:100 dilution (ABCAM) was analyzed. Visualization was carried out with the use of peroxidase – tetrachloride 3,3- Diaminobenzidine (DAB).

The intensity of the reaction was measured on a scale from 0–3 (0 – lack, 1 – weak, 2 – moderate, 3 – strong). 0 and 1 were considered 'negative', 2 and 3 'positive'.

Statistical analysis

The results were statistically analyzed with SPSS version 14 software, the *p* values were calculated with Fisher's exact test. $p<0.05$ was considered statistically significant.

Results

The study group included 19 women, 8 (42.2%) pre-menopausal and 11 (57.8%) post-menopausal, diagnosed with clear cell ovarian carcinoma. 9 patients were FIGO stage I (47.3%) and 10 were FIGO stage II (52.6%). 7 tumors were classified as G1 (36.8%) and 12 as G2 (63.2%). The results are presented in table I.

Table I. Podoplanin expression with reference to clinical tumor features.

Characteristics	N (number)	Podoplanin expression % positive N (%)	negative N (%)	p *
stage of advancement according to FIGO				
I	9	3 (33.3)	6 (66.7)	
II	10	7 (70)	3 (30)	0.179
maturity of tumor				
G1	7	2 (28.6)	5 (71.4)	
G2	12	8 (66.7)	4 (33.3)	0.170

Fisher's exact test

Table II. Cell localization of podoplanin in patients with OCCC.

	Podoplanin expression	lack	weak	total	moderate	strong	total
Cell localization	membrane	0	1	1	2	1	3
	cytoplasmic-membrane	0	2	2	4	3	7

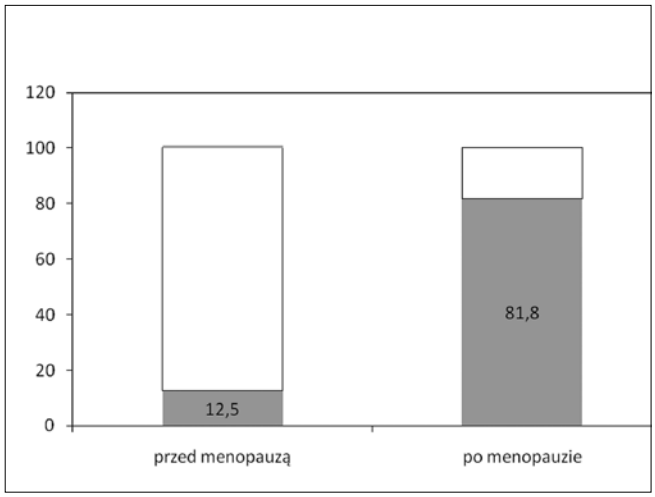

Figure 1. Podoplanin expression with reference to patient age (%).

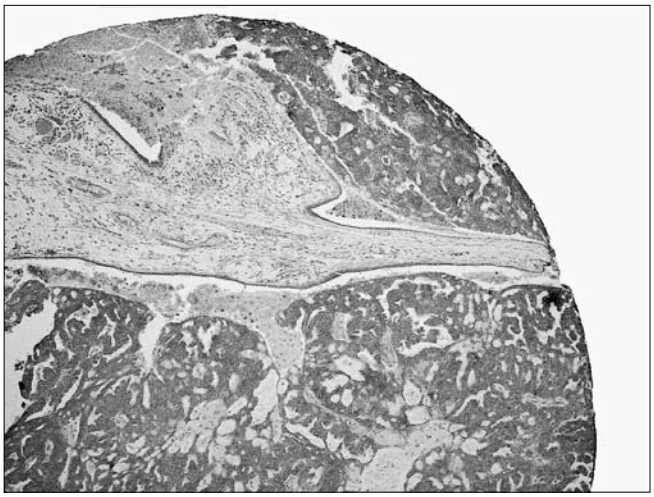

Photo 1. High podoplanin expression within the cytoplasm and cell membrane in clear cell ovarian carcinoma (Dako EnVision™+/HRP; magnified 5x).

Podoplanin expression

Out of the 19 analyzed cases of clear cell carcinoma, moderate or strong podoplanin expression was observed in 10 patients, what accounted for 52%. (Photo 1).

It was weak in 3 cases (16%). Membrane expression was found in 4 (31%) and cytoplasmic-membrane in 9 cases (69%). (Table II). No differences in podoplanin expression in different stages of advancement and maturity of the tumor were found. (Table I).

There were differences, however, in the expression of podoplanin with regard to menopausal status. Podoplanin expression was more often observed (p=0.005) in the group of post-menopausal (81.8%) than pre-menopausal patients (12.5%). (Figure 1).

Discussion

Presence of podoplanin expression was found in a significant number of cases (52%) of ovarian clear cell carcinoma. Due to the rare incidence (<5% of all EOC) of such malignancy, when comparing to other histologic subtypes, the study group was limited in number (19 cases). Regardless of that fact, the obtained results indicate that podoplanin may in fact play a significant role in the lymphangiogenesis of clear cell ovarian carcinoma. Oe et al., have drawn similar conclusions after having conducted their study in a slightly larger group of patients (22 cases – 54.5% of the OCCC women demonstrated podoplanin expression). So far their findings have been the only report on the role of podoplanin in clear cell ovarian carcinoma. In their research they noted statistically significantly higher podoplanin expression in

OCCC when compared to other histological subtypes of ovarian cancer. It correlated with tumor malignancy and poor response to therapy. Therefore, they claim the protein to be a marker differentiating clear cell carcinoma from other epithelial ovarian cancers [10]. The number of cases of OCCC with positive podoplanin expression confirmed by our study enables the authors to agree with the conclusions of Oe et al. It is necessary to monitor the results of the treatment of patients with OCCC in order to determine whether that carcinoma occurs in two possible groups (podoplanin+ and podoplanin-) of different courses of the disease. Bearing in mind the mechanisms of podoplanin activity, its increased expression in OCCC might explain that fact that patients with clear cell carcinoma are more prone to thrombosis than patients diagnosed with other types of ovarian cancer [12].

It has not been conclusively resolved whether one or many mechanisms of podoplanin have the pathogenic influence on OCCC, at what stage of OCCC carcinogenesis the role of podoplanin is the key one, whether it is the reason or the cause of the molecular cancerous processes undergoing within a cell. What has been known is that high expression of podoplanin is induced during cell transformation dependant on the tyrosine kinase Src. Shen et al., concluded on the basis of their *in vitro* tests that increased expression of podoplanin was, to a large degree, responsible for higher migration and invasiveness of transformed cells [13]. It is yet another proof that might explain the different and aggressive phenotype of OCCC, particularly in cases with increased expression of podoplanin.

Suzuki-Inoue et al., demonstrated in tests on mice that using antibodies to block podoplanin protects from tumor metastasis [6]. Other authors proved that transfection and ectopic expression of CD9 inhibited platelets aggregation, induced by Aggrus factor, and high expression of podoplanin in lung tumor. As a result, suppression of metastases occurred [14]. A similar effect was reported by Cortez et al., who lowered high expression of podoplanin by inducing the expression of two microRNAs (miR-29b and miR-125A) in glioblastoma and suppressed tumor invasion [15].

Taking into account the increased expression of podoplanin observed by the authors of the present work and by Oe [10], the abovementioned mechanisms might be used in treatment of patients diagnosed with clear cell ovarian carcinoma. Increased expression of podoplanin might also be a significant factor for allowing a more effective differentiation of OCCC in the highly heterogeneous group of epithelial ovarian tumors.

In our study podoplanin expression was not statistically significant with regard to clinical features (FIGO, tumor maturity) ($p>0.05$). Its expression, however, was more characteristic ($p= 0,005$) in case of post-menopausal women when compared to pre-menopausal ones, what might explain more aggressive progression of the tumor and worse prognosis for post-menopausal women. These findings are confirmed by tests in epithelial ovarian cancer group. The course of the disease, prognosis and response to therapy were significantly better in the group of pre-menopausal patients [16, 17].

In our work we have noticed almost 69% cases of cytoplasmic-membrane podoplanin cell localization. Physiologically podoplanin is located in cell membrane. Its accumulation in the cytoplasm may lead to other, so far undiscovered, anomalies on the molecular level, for example signal transduction pathway. Clinical application of podoplanin as a marker might be broadened if the influence of its cell localization on the function was explained.

Due to the fact that clear-cell ovarian carcinoma is relatively rare and heterogeneous in nature (papillary, tubulocystic, solid), selecting statistically representative groups of patients presents a considerable challenge. Therefore, the results of the authors of this article are preliminary and aim at presenting a certain tendency and at attracting attention to the possibility of using podoplanin in clinical clear cell cancer diagnosis.

Conclusions

Increased expression of podoplanin is typical of clear cell ovarian cancer, especially for post-menopausal women with OCCC.

References

1. Wicki A, Christofori G. The potential role of podoplanin in tumour invasion. *Br J Cancer.* 2007, 96,1-5.
2. Raica M, Cimpean A, Ribatti D. The role of podoplanin in tumor progression and metastasis. *Anticancer Res.* 2008, 28, 2997-3006.
3. Wicki A, Lehembre F, Wick N, [et al.]. Tumor invasion in the absence of epithelial–mesenchymal transition: podoplanin-mediated remodeling of the actin cytoskeleton. *Cancer Cell.* 2006, 9, 261–272.
4. Kunita A, Kashima T, Morishita Y, [et al.]. The platelet aggregation-inducing factor aggrus/podoplanin promotes pulmonary metastasis. *Am.J Pathol.* 2007, 170, 1337–1347.
5. Ozaki Y, Suzuki-Inoue K, Inoue O. Novel interactions in platelet biology: CLEC-2/podoplanin and laminin/GPVI. *J Thromb Haemost.* 2009, 1, 191-194.
6. Suzuki-Inoue K, Kato Y, Inoue O, [et al.]. Involvement of the snake toxin receptor CLEC-2, in podoplanin-mediated platelet activation, by cancer cells. *J Biol Chem.* 2007, 282, 25993–26001.
7. Pectasides D, Pectasides E, Psyrri A, Economopoulos T. Treatment issues in clear cell carcinoma of the ovary: a different entity? *Oncologist.* 2006, 11, 1089-1094.
8. Sugiyama T, Kamura T, Kigawa J, [et al.]. Clinical characteristics of clear cell carcinoma of the ovary: a distinct histologic type with poor prognosis and resistance to platinum-based chemotherapy. *Cancer.* 2000, 88, 2584–2589.
9. Takano M, Kikuchi Y, Yaegashi N, [et al.]. Clear cell carcinoma of the ovary: a retrospective multicentre experience of 254 patients with complete surgical staging. *Br J Cancer.* 2006, 94, 1369-1374.
10. Oe S, Hasegawa K, Nagase S. [et al.]. Expression of podoplanin in epithelial ovarian carcinomas and its potential as a marker for clear cell adenocarcinoma. *Int J Gynecol Pathol.* 2010, 29, 405-410.
11. Lesiak A, Słowik-Rylska M, Jochymski C, [i wsp.]. Udział ekspresji cyklin w patogenezie odmiany powierzchownej i guzkowej raka podstawnokomórkowego. *Post Dermatol Alergol.* 2008, 4, 157–163.
12. Duska L, Garrett L, Henretta M, [et al.]. When 'never-events' occur despite adherence to clinical guidelines: the case of venous thromboembolism in clear cell cancer of the ovary compared with other epithelial histologic subtypes. *Gynecol Oncol.* 2010, 116, 374-377.
13. Shen Y, Chen C, Ichikawa H, Goldberg G. SRC induces podoplanin expression to promote cell migration. *J Biol Chem.* 2010, 285, 9649-9656.
14. Nakazawa Y, Sato S, Naito M, [et al.]. Tetraspanin family member CD9 inhibits Aggrus/podoplanin-induced platelet aggregation and suppresses pulmonary metastasis. *Blood.* 2008, 112, 1730-1739.
15. Cortez M, Nicoloso M, Shimizu M, [et al.]. miR-29b and miR-125a regulate podoplanin and suppress invasion in glioblastoma. *Genes Chromosomes Cancer.* 2010, 49,981-990.
16. Chan J, Loizzi V, Lin Y, [et al.]. Stages III and IV invasive epithelial ovarian carcinoma in younger versus older women: what prognostic factors are important? *Obstet Gynecol.* 2003, 102, 156-161.
17. Pectasides D, Fountzilas G, Aravantinos G, [et al.]. Epithelial ovarian carcinoma in younger vs older women: is age an independent prognostic factor? The Hellenic Oncology Cooperative Group experience. *Int J Gynecol Cancer.* 2007,17, 1003-1010.

The paper was financed by the KBN grant No. N N407 172434. The equipment used was purchased with funds from 'The equipment of Innovative laboratories doing research on new medicines used in the therapy of civilization and neoplastic diseases' Project, part of the Development of Eastern Poland Operational Program 2007-2013, Priority Axis and Modern Economy, Operations I.3 Innovation Promotion.