The immunohistochemical expression of CD24 and CD171 adhesion molecules in borderline ovarian tumors

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CD24 and CD171 are cell adhesion proteins, which have been shown to be overexpressed in several carcinomas and to be associated with a poor clinical outcome. Our aim was to determine the expression of these two adhesion molecules in ovarian borderline neoplasms. We investigated 50 ovarian borderline tumors (serous, mucinous and endometrioid) as well as 29 benign cystadenomas and 25 carcinomas, which were used as controls. Paraffin sections were stained immunohistochemically for CD24 and CD171, and their expression was recorded in a semi-quantitative manner.
In normal epithelium and benign ovarian cystadenomas both the CD24 and CD171 expression was negative to low, while their expression was significantly increased in borderline and malignant ovarian tumors. High-grade carcinomas, and carcinomas with metastases to the omentum presented considerably higher CD24 expression than low-grade carcinomas, and carcinomas without metastases. In addition, a few borderline and many malignant tumors presented cytoplasmic CD24 immunoreactivity, whereas all benign and most borderline tumors showed apical localization of this molecule. In conclusion, borderline tumors and carcinomas of the ovary present increased expression of CD24 and CD171 in relation to their benign counterparts, as is the case in malignant tumors of other organs. Change of staining pattern of CD24 (apical to cytoplasmic) apparently relates to a more aggressive phenotype.

Key words: borderline ovarian tumors, ovarian cancer, CD24, CD171, cell adhesion molecules.

Introduction

Ovarian tumors grow silently, in many instances attaining very large size, and their behavior usually can only be predicted postoperatively after histological evaluation. In this regard, most of them fall into one out of three categories, namely benign, borderline or malignant. This scheme is especially applicable for the most common of the ovarian tumors, which derive from the surface epithelium. Still, classic histological classification is not always adequate for proper and effective management and prognosis. Currently, molecular markers have become quite promising in tumor diagnostics and therapeutics. Among them recognition and adhesion molecules seem to play an important role in carcinogenesis and tumor progression.

CD24 is a sialoglycoprotein that is anchored to the cell surface by a glycosyl phosphatidylinositol linkage. It was originally described as a B-cell specific marker, which is expressed in the early stages of B-cell development, as well as in several solid tumors, including breast, colon, prostate, periampullary, and renal cell carcinomas, and neuroblastoma [1-4]. CD171, also known as L1CAM, is a 200-kDa transmembrane glycoprotein. It is a neuronal cell adhesion molecule involved in axon guidance and cell migration [5] with a strong implication in treatment-resistant cancers [6-8]. L1CAM belongs to the immunoglobulin superfamily

of recognition molecules, and participates in heterophilic interactions with other adhesion molecules such as laminin, integrins, proteoglycans and CD24. It is also expressed by hematopoietic cells and a variety of tumor cell lines [2, 9]. We undertook the task of investigating these molecules in ovarian neoplasms, focusing on the borderline tumor category, since literature concerning the expression of these molecules in ovarian cancer is still limited [10-16].

Material and methods

Our material included 50 borderline ovarian tumors (33 serous, 16 mucinous and 1 endometrioid). In addition, we studied 29 ovarian cystadenomas (10 serous, 10 mucinous cystadenomas and 9 endometrioid cysts), and 25 ovarian invasive carcinomas (10 serous, 5 mucinous, and 10 endometrioid), which were used as controls. Three patients with borderline tumors (all of them serous type) had peritoneal implants, and 6 patients with ovarian carcinoma (4 serous and 2 endometrioid) had metastases to the omentum.

Immunohistochemical technique

Immunohistochemical detection was performed on formalin fixed, paraffin embedded tissue sections. The following monoclonal antibodies were applied: CD24 (dilution 1 : 20, clone 5G3, eBioscience, San Diego, CA, USA) and CD171 (dilution 1 : 20, clone SN3, NeoMarkers, Fremont, CA, USA). A standardized automated (Nexes, Ventana, Tucson, Arizona, USA), streptavidin-biotin immunohistochemical detection system was used (I-VIEW Paraffin DAB; Ventana). Normal tonsils were used as a positive control for CD24, and normal peripheral nerves were used as a positive control for CD171. A negative control for immunostaining was carried out by replacing the primary antibody with non-immune rabbit serum.

Immunohistochemistry scoring

Both the intensity and the extent of immunostaining were evaluated for each antibody, in a semiquantitative way. The intensity of immunostaining was graded on a scale of 0 to 3, where 0 – negative reaction, 1 – weak, 2 – moderate and 3 – strong reaction. The extent of immunoreactivity was expressed as the percentage of immunoreactive cells, and was recorded separately for each of the intensity categories (0 to 3). The sum of the products for each intensity category (intensity grade X percentage of positive cells) was the final score of each case (score range: 0 to 300). In order to interpret better our results, we regarded our cases as negative (score = 0), mildly positive (score = 1-100), moderately positive (score = 101-200), and strongly positive (score = 201-300).

Statistical analysis

IBM SPSS version 17 for Windows and Microsoft Excel version 10 for Windows were used for statistical analysis of the collected data. Student's t-test was performed for comparison of the mean values between different categories of tumors.

Results

The mean age of the patients with benign cystadenomas was 39.31 ±15.30, the mean age of the patients with borderline tumors was 41.11 ±12.14, and the mean age of the patients with malignant tumors was 57.91 ±14.70. The results of CD24 and CD171 immunostaining of ovarian tumors are shown in Table I.

CD24 immunoreactivity

The cellular staining pattern was either apical (more commonly) or both membranous and cytoplasmic. 1) Apical staining: Normal ovarian epithelium showed no immunoreaction, benign cystadenomas had negative to weak staining intensity, while borderline tumors and invasive carcinomas generally showed much stronger immunostaining (Fig. 1). The difference between the benign tumors on the one hand, and the borderline and malignant tumors on the other hand, almost reached statistical significance (benign vs. borderline tumors $p = 0.06$, benign vs. malignant tumors $p = 0.09$, borderline vs. malignant $p = 0.46$). No significant differences were observed between the different histological types (serous vs. mucinous vs. endometrioid) except for the malignant category of tumors, where serous carcinomas had on average almost double the scores of mucinous carcinomas ($p = 0.06$). Borderline tumors without peritoneal implants did not differ significantly in their staining intensity from cases with peritoneal implants. However, low-grade carcinomas had much lower scores than high-grade carcinomas ($p = 0.05$), and carcinomas with peritoneal metastases had on average more than double the staining scores of cases without peritoneal metastases ($p = = 0.08$). 2) Membranous and cytoplasmic staining: 3/50 (6%) borderline tumors, 5/7 (71.43%) serous carcinomas, and 1/8 (12.50%) endometrioid ovarian adenocarcinomas presented CD24 cytoplasmic staining, which was accentuated in a membranous pattern along the circumference of tumor cells (Fig. 2). Two out of the five cases (40%) with serous carcinoma showing cytoplasmic CD24 staining pattern had metastases to the omentum.

CD171 immunoreactivity

The staining pattern of CD171 was always membranous (Fig. 3). The lowest immunoreactivity scores

Table I. CD24 and CD171 immunostaining in benign, borderline and malignant epithelial ovarian tumors (mean ± standard deviation)

Tumor type	Number	CD24	CD171
Benign	29	28.97 ±31.83	67.24 ±46.36
serous cystadenomas	10	39.00 ±33.48	72.00 ±45.51
mucinous cystadenomas	10	32.00 ±26.58	63.00 ±48.55
endometriotic cysts	9	14.44 ±31.66	66.67 ±47.14
Borderline	50	79.20 ±60.57	128.80 ±53.82
serous	33	79.39 ±50.62	122.12 ±52.95
mucinous	16	77.50 ±58.63	141.25 ±54.39
endometrioid	1	100.00	150.00
Peritoneal implants			
absent	47	79.57 ±61.04	129.79 ±55.19
present	3	73.33 ±64.29	113.33 ±23.09
Malignant	25	73.20 ±79.88	130.80 ±49.15
serous carcinomas	10	89.00 ±85.30	146.00 ±57.97
mucinous carcinomas	5	46.00 ±61.89	120.00 ±27.39
endometrioid carcinomas	10	71.00 ±81.29	121.00 ±45.71
Grade			
I (low)	15	63.27 ±48.31	131.11 ±41.43
II & III (high)	10	131.00 ±64.46	130.73 ±64.35
Metastases			
absent	19	52.11 ±61.61	129.47 ±40.89
present	6	140.00 ±99.60	135.00 ±74.50

were recorded in normal ovaries and benign ovarian tumors, while the highest scores were found in malignant tumors (Table I). However, none of these differences reached statistical significance (benign vs. borderline, $p = 0.10$; benign vs. malignant, $p = 0.09$; borderline vs. malignant $p = 0.49$). It is noteworthy that only one case was strongly positive for CD171, a serous carcinoma presenting extensive peritoneal metastases. The tumor cells showed increased immunoreactivity especially at the invasive front of the tumor. There were no significant differences between tumors of different histological type, between borderline tumors with and without peritoneal implants, low-grade and high-grade carcinomas, and between malignant tumors with and without metastases to the omentum.

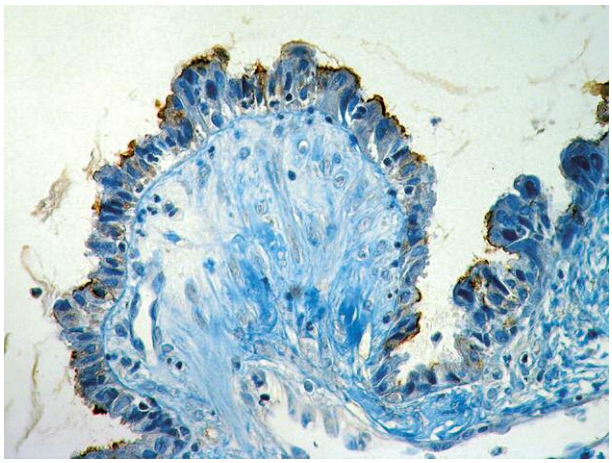

Fig. 1. Serous borderline tumor of the ovary showing apical immunohistochemical expression of CD24 (DAB, hematoxylin, magnification 400×)

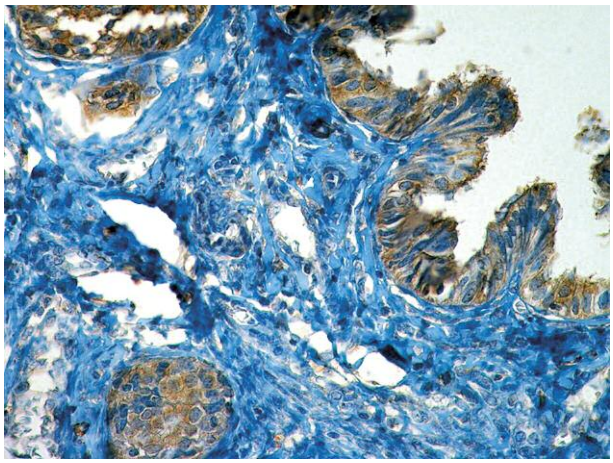

Fig. 2. Serous carcinoma of the ovary showing cytoplasmic and membranous immunohistochemical expression of CD24 (DAB, hematoxylin, magnification 400×)

Discussion

CD24 is a ligand of P-selectin, an adhesion receptor on activated endothelial cells and platelets, and thus might contribute to the metastasizing capacities of CD24-expressing tumor cells [17, 18]. Immunohistochemical expression of CD24 has been investigated in various carcinomas, including those of the stomach, colon, gallbladder, breast, endometrium, pancreas, and the lung [1, 19, 20]. In general, malignant tumors, especially those of higher histological grade and those with metastatic disease, tend to present stronger immunoreaction, while CD24 expression has been associated with survival and prognosis. Previous studies in ovarian cancer cases have shown that overexpression of CD24 is an independent factor associated with tumor metastasis, and poor survival [10-14]. In this study the expression of CD24 was considerably higher in borderline tumors and ovarian carcinomas than normal ovaries and benign neoplasms. However, the intensity scores of this marker were very close when considering borderline tumors and carcinomas. This implies that the latter two categories may share some molecular pathways. In addition, high-grade carcinomas and carcinomas with metastases to the omentum had considerably higher CD24 scores, confirming the results of previous reports [10-14]. The polarized apical expression of CD24 was maintained in benign cystadenomas and most borderline tumors, while progression to carcinoma made prominent a non-polarized, cytoplasmic expression of CD24, an observation made by other authors as well [10-13]. This change of expression pattern may be due to overproduction, disturbance of protein distribution or degradation within the cell [11]. Accumulative data from our results and those of the literature [10-13] support the view that cytoplasmic expression of CD24 in a borderline or a malignant ovarian tumor may suggest a more aggressive phenotype.

Overexpression of CD171 has been shown to promote tumor cell growth, cell motility and migration as well as angiogenesis [7, 8]. Several reports suggest that L1CAM is also involved in apoptosis resistance [6]. Tumors of various sites express this molecule, including neoplasms of the female genital tract, colon, stomach, pancreas, skin, and the kidneys [6-8, 21-23]. Expression is often found at the invasive front of primary tumors [24], strongly supporting a role for L1CAM in metastasis. Increased expression of L1CAM has been correlated with lymph node metastases, advanced tumor stage and reduced patient survival [24, 25]. In our material CD171 expression was more prominent in borderline and malignant tumors than benign cystadenomas, but it did not relate to tumor grade or stage of the disease. Still other authors have found an association of CD171 expression with tumor stage and survival in ovarian cancer [15, 16], and so further investigation is warranted.

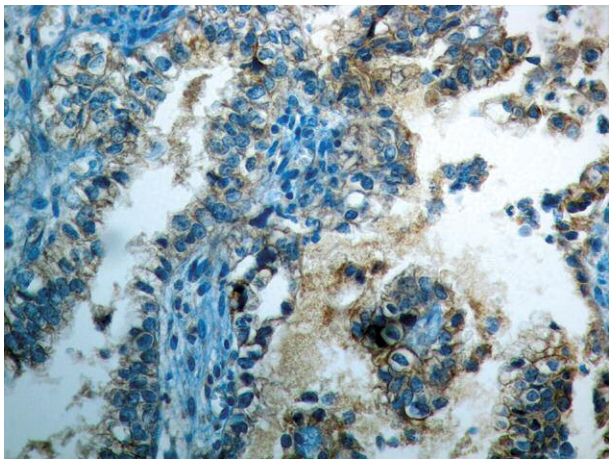

Fig. 3. Membranous immunohistochemical staining for CD171 in a serous borderline tumor of the ovary (DAB, hematoxylin, magnification 400×)

In conclusion, CD24 and CD171 expression in the ovaries seems to be associated with tumor malignancy, as is the case in tumors of other organs, and may indicate that a neoplasm has attained a more aggressive phenotype. If such a correlation is established, it may have therapeutic implications, and potentially lead to the development of targeted therapies with a better patient response [24, 29, 30].

The authors declare no conflict of interest.

References

1. Lim SC, Oh SH. The role of CD24 in various human epithelial neoplasias. Pathol Res Pract 2005; 201: 479-486.
2. Zetter BR. Adhesion molecules in tumor metastasis. Semin Cancer Biol 1993; 4: 219-229.
3. Wang W, Wang X, Peng L, et al. CD24-dependent MAPK pathway activation is required for colorectal cancer cell proliferation. Cancer Sci 2010; 101: 112-119.
4. Baumhoer D, Riener MO, Zlobec I, et al. Expression of CD24, P-cadherin and S100A4 in tumors of the ampulla of Vater. Mod Pathol 2009; 22: 306-313.
5. Zhang Y, Yeh J, Richardson PM, Bo X. Cell adhesion molecules of the immunoglobulin superfamily in axonal regeneration and neural repair. Restor Neurol Neurosci 2008; 26: 81-96.
6. Sebens Müerköster S, Werbing V, Sipos B, et al. Drug-induced expression of the cellular adhesion molecule L1CAM confers antiapoptotic protection and chemoresistance in pancreatic ductal adenocarcinoma cells. Oncogene 2007; 26: 2759-2768.
7. Doberstein K, Wieland A, Lee SB, et al. L1-CAM expression in ccRCC correlates with shorter patients survival times and confers chemoresistance in renal cell carcinoma cells. Carcinogenesis 2011; 32: 262-270.
8. Yoon H, Min JK, Lee DG, Kim DG, Koh SS, Hong HJ. L1 cell adhesion molecule and epidermal growth factor receptor activation confer cisplatin resistance in intrahepatic cholangiocarcinoma cells. Cancer Lett 2012; 316: 70-76.
9. Moos M, Tacke R, Scherer H, Teplow D, Früh K, Schachner M. Neural adhesion molecule L1 as a member of the immunoglobulin superfamily with binding domains similar to fibronectin. Nature 1998; 334: 701-703.

10. Choi YL, Kim SH, Shin YK, et al. Cytoplasmic CD24 expression in advanced ovarian serous borderline tumors. Gynecol Oncol 2005; 97: 379-386.
11. Kristiansen G, Denkert C, Schlüns K, Schlüns K, Dahl E, Pilarsky C, Hauptmann S. CD24 is expressed in ovarian cancer and is a new independent prognostic marker of patient survival. Am J Pathol 2002; 161: 1215-1221.
12. Zhu J, Zhang G, Lu H. CD24, COX-2, and p53 in epithelial ovarian cancer and its clinical significance. Front Biosci (Elite Ed) 2012; 4: 2745-2751.
13. Aktaş IY, Buğdayci M, Usubütün A. Expression of p16, p53, CD24, EpCAM and Calretinin in Serous Borderline Tumors of the Ovary. Turk Patoloji Derg 2012; 28: 220-230.
14. Biade S, Marinucci M, Schick J, et al. Gene expression profiling of human ovarian tumours. Br J Cancer 2006; 95: 1092-1100.
15. Fogel M, Huszar M, Altevogt P, et al. L1 (CD171) as a novel biomarker for ovarian and endometrial carcinomas. Expert Rev Mol Diagn 2004; 4: 455-462.
16. Daponte A, Kostopoulou E, Kollia P, et al. L1 (CAM) (CD171) in ovarian serous neoplasms. Eur J Gynaecol Oncol 2008; 29: 26-30.
17. Sammar M, Aigner S, Hubbe M, Schirrmacher V, Schachner M, Vestweber D, Altevogt P. Heat-stable antigen (CD24) as ligand for mouse P-selectin. Int Immunol 1994; 6: 1027-1036.
18. Aigner S, Ramos CL, Hafezi-Moghadam A, Lawrence MB, Friederichs J, Altevogt P, Ley K. CD24 mediates rolling of breast carcinoma cells on P-selectin. FASEB J 1998; 12: 1241-1251.
19. Jacob J, Bellach J, Grützmann R, et al. Expression of CD24 in adenocarcinomas of the pancreas correlates with higher tumor grades. Pancreatology 2004; 4: 454-460.
20. Kim KH, Choi JS, Kim JM, et al. Enhanced CD24 expression in endometrial carcinoma and its expression pattern in normal and hyperplastic endometrium. Histol Histopathol 2009; 24: 309-316.
21. Huszar M, Moldenhauer G, Gschwend V, Ben-Arie A, Altevogt P, Fogel M. Expression profile analysis in multiple human tumors identifies L1 (CD171) as a molecular marker for differential diagnosis and targeted therapy. Hum Pathol 2006; 37: 1000-1008.
22. Meier F, Busch S, Gast D, et al. The adhesion molecule L1 (CD171) promotes melanoma progression. Int J Cancer 2006; 119: 549-555.
23. Allory Y, Matsuoka Y, Bazille C, et al. The L1 cell adhesion molecule is induced in renal cancer cells and correlates with metastasis in clear cell carcinomas. Clin Cancer Res 2005; 11: 1190-1197.
24. Zecchini S, Bianchi M, Colombo N, et al. The differential role in L1 in ovarian carcinoma and normal ovarian surface epithelium. Cancer Res 2008; 68: 1110-1118.
25. Kaifi JT, Reichelt U, Quaas A, et al. L1 is associated with micrometastatic spread and poor outcome in colorectal cancer. Mod Pathol 2007; 20: 1183-1190.
26. Su D, Deng H, Zhao X, et al. Targeting CD24 for treatment of ovarian cancer by short hairpin RNA. Cytotherapy 2009; 11: 642-652.
27. Schäfer H, Dieckmann C, Korniienko O, et al. Combined treatment of L1CAM antibodies and cytostatic drugs improve the therapeutic response of pancreatic and ovarian carcinoma. Cancer Lett 2012; 319: 66-82.

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