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Original Article

Overexpression of CD44 and EpCAM may be associated with the initiation and progression of epithelial ovarian cancer

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Abstract: Objective: Cluster of differentiation 44 (CD44) and epithelial cell adhesion molecule (EpCAM) play an essential role in cancer initiation and progression via inducing tumor cells proliferation, differentiation, invasion and migration. This study was to investigate the protein expression levels of CD44 and EpCAM in epithelial ovarian cancer and to evaluate the correlation between expression of these markers and the clinical pathological features, as well as the correlation between CD44 and EpCAM expression. Methods: The expression of CD44 and EpCAM in 50 formalin-fixed paraffin-embedded (FFPE) human epithelial ovarian cancer specimens and 15 FFPE normal ovary specimens was examined. Clinical and pathological parameters were collected, including age, clinical stage, status of lymph node metastasis, histological type and histological grade. The Statistical Package for Social Sciences version 17.0 was used for all statistical analyses. Results: The levels of CD44 and EpCAM expression in epithelial ovarian cancer were increased compared with normal ovary tissues. Moreover, the increased CD44 and EpCAM protein expression were closely related with International Federation of Gynecology and Obstetrics (FIGO) stage and histological grade, lymph node metastasis, whereas had no statistically significant association with age, histological type. A significantly positive correlation between CD44 and EpCAM expression was detected in epithelial ovarian carcinoma tissues. Conclusions: Overexpression of CD44 and EpCAM may be involved in the pathogenesis of epithelial ovarian cancer and associated with the initiation and progression of epithelial ovarian cancer. These results further indicated that CD44-EpCAM-targeted therapy might be a potential strategy in epithelial ovarian cancer.

Keywords: Epithelial ovarian cancer, CD44, EpCAM, immunohistochemistry

Introduction

Ovarian carcinoma is the most frequent gynecologic malignancy resulting in cancer-related death. Epithelial ovarian cancer constitutes 85%-90% cases of ovarian carcinoma. According to GLOBOCAN estimates, an estimated 238,700 new ovarian cancer cases and 151,900 deaths occurred in 2012 worldwide [1]. The lack of early stages specific symptoms contribute to the high mortality of ovarian carcinoma. As a consequence, more than 70% patients are diagnosed at advanced stages when it presents extensive local invasion and intraperitoneal metastasis. In spite of the cytoreduction surgery and platinum-paclitaxel chemotherapy as the standard treatment have been performed for advanced stage disease, the majority patients finally relapse and the 5-year overall survival rate only 45% [2]. Therefore, it is necessary to investigate the biological behavior of ovarian carcinoma to explore the molecular target-directed therapies which inhibit the local invasion and distant metastasis and represent a new treatment modality.

Cluster of differentiation 44 (CD44), initially identified as a leukocyte antigen, is a single-span transmembrane glycoprotein with three functional domains, including an intracellular domain, an extracellular domain and a transmembrane domain [3]. CD44 is encoded by the highly conserver gene about 60 kb of length located in chromosome 11 in human. The CD44

gene is composed of 20 exons and 19 introns [4]. CD44 is the principal receptor for hyaluronan (HA). The binding of HA with CD44 induces tumor cell proliferation, differentiation, invasion, and migration, leading to the progression and metastasis of tumors [5]. In addition, it has been reported that CD44 plays a critical role in promoting chemotherapy resistance in cancer cells and animal models [6, 7].

Epithelial cell adhesion molecule (EpCAM, also known as CD326) was initially identified in 1979 as a predominant antigen in human colon carcinoma tissue. EpCAM is a type I transmembrane glycoprotein, calcium-independent, homophilic, epithelial-specific intercellular adhesion molecule [8], with a molecular weight of 39-42 kDa, and contains a large extracellular domain, a single transmembrane region, and a short intracellular domain of 26 amino acids [9]. EpCAM is not only expressed in human normal epithelium, with the exception of squamous epithelium and of specific epithelial cells of adult hepatocytes and keratinocytes, but also abundantly expressed in various human epithelial neoplasms [10]. It is more frequently positive expression in tumor cells than in the normal epithelia. In addition to mediating cell adhesion, enough evidence revealed that EpCAM also participate in cellular signaling, cell proliferation, migration, invasion and differentiation [11].

As overexpression of CD44 and EpCAM plays a dominant role in development and progression of human cancers, we hypothesized that CD44 and EpCAM might have a correlation in epithelial ovarian cancer progression. In this study we evaluated the status of CD44 and EpCAM expression in epithelial ovarian cancer tissues by immunohistochemistry. Furthermore, we analyzed the association of protein expression with clinicopathological features and the possible relevance between CD44 and EpCAM, which would provide a promising joint molecular therapeutic target for epithelial ovarian carcinoma.

Materials and methods

Tissue specimens

50 formalin-fixed paraffin-embedded (FFPE) epithelial ovarian cancer samples, 15 FFPE normal ovarian epithelial tissue samples which obtained from the normal ovaries during surgery for other gynecological diseases were collected in this study. The screening specimens were obtained from April 2013 to December 2015 in the Department of Pathology of the Second Hospital of Jilin University. None of the epithelial ovarian cancer patients had taken chemotherapy or radiotherapy prior to their surgery. All hematoxylin-eosin slides were reviewed by two pathologists to evaluate histological grade and histological type according to World Health Organization (WHO) criteria to achieve a consensus diagnosis. The patient signed informed consent and the present study was carried out with the approved of the Medical Ethics Committee of the Second Hospital of Jilin University.

Immunohistochemistry

FFPE tissue specimens were cut into 4 μm-thick sections, mounted and baked at 60°C for 1 hour. The tissue sections were dewaxed in xylene, rehydrated in graded ethanol, and incubated in 3% H_2O_2 for 5 minutes to block endogenous peroxidase activity. Antigen retrieval was performed by heating the slides in a pressure cooker in 10 mM citric acid buffer (pH 6.0) at 100°C for 15 minutes. The slides were rinsed in 0.1 M Tris-HCl (PBS, pH 7.6) for 5 minutes 3 times and then immersed in PBS containing 10% goat serum to block non-specific binding for 30 minutes at room temperature. The slides were incubated overnight at 4°C with the primary antibody against either CD44 (rabbit anti-human monoclonal antibody, 1:100, Abcam, MA, USA) or EpCAM (mouse anti-human monoclonal antibody, 1:200, Abcam, MA, USA). After washing in PBS for 5-minute 3 times, slides were incubated with horseradish-peroxidase-labeled goat anti-mouse/rabbit IgG/HRP conjugated polymer (PV-6000, ZSGB-BIO) for 20 minutes at 37°C. After three 5-minute washes in PBS, staining was visualized by immersed slides in the solution of 3,3-diaminobenzidine tetrahydrochloride with hydrogen peroxidase. Subsequently the slides were rinsed with distilled water and counterstained with hematoxylin solution, dehydrated, cleared and sealed. Negative control slides were processed in the same way except that the primary antibody was substituted by phosphate buffered saline solution.

Table 1. Association analyses between expression of EpCAM and CD44 and the clinical pathological characteristics in epithelial ovarian cancer

Characteristic	*n*	EpCAM Positive (%)	*P*	CD44 Positive (%)	*P*
Age					
≥55	27	23 (85.2)	0.523	17 (63)	0.869
<55	23	17 (73.9)		15 (65.2)	
Histological type					
Serous	36	30 (83.3)	0.466	22 (61.1)	0.636
Mucinous	9	7 (77.8)		7 (77.8)	
Endometrioid	5	3 (60)		3 (60)	
Differentiation					
Well-moderately	30	20 (66.7)	0.03	15 (50)	0.012
Poorly	20	20 (100)		17 (85)	
FIGO stage					
I-II	25	17 (68)	0.034	12 (48)	0.018
III-IV	25	23 (92)		20 (80)	
Lymphatic metastasis					
No	27	18 (66.7)	0.028	13 (48.1)	0.011
Yes	23	22 (95.7)		19 (86.3)	

Evaluation procedures

EpCAM and CD44 immunostaining were seen in the cell membrane of epithelial ovarian cancer. All slides were assessed independently by 2 investigators who were blinded to clinicopathologic information. Immunostaining was evaluated in a series of randomly selected five high-power fields (200 × magnification) and 100 tumor cells were counted in each field. Staining intensity was graded as follows: 0, no staining; 1, weak staining; 2, moderate staining; 3, strong staining. The staining extent was graded according to the proportion of positive tumor cells as follows: score 0, 0-5% positive tumor cells; 1, 6-25% positive tumor cells; 2, 26-50% positive tumor cells; 3, 51-75% positive tumor cells; 4, 76-100% positive tumor cells. The staining intensity score was multiplied with the staining extent score, resulting in the semiquantitative immunoreactivity score that indicated the expression level: 0-2, negative; 3-4, weak positive; 6-8, moderate positive; 9-12, strong positive.

Statistical analyses

Statistical analysis was carried out using the Statistical Package for Social Sciences version 17.0 (SPSS 17.0, IBM, Chicago, IL, USA). The Chi-square test or Fisher's exact test was used to evaluate the correlation of EpCAM or CD44 expression with clinicopathologic parameters. The correlation between EpCAM expression and CD44 expression was investigated using Spearman correlation analysis. For all comparisons, $P<0.05$ was considered to be statistically significant.

Results

Clinical pathology information

The clinicopathological characteristics of patients were gathered from the patients' medical records and pathology reports and are shown in **Table 1**. The diagnosis of histological type and grade were performed according to the Classification of Ovarian Cancer (WHO 2004). Among 50 cases of epithelial ovarian carcinoma, 36 cases were serous cystadenocarcinoma, 9 cases were mucinous cystadenocarcinoma and 5 cases were endometrioid carcinoma. Regarding tumor histological grade (tumor differentiated), 15 cases were well differentiated (Grade 1), 15 cases were moderately differentiated (Grade 2) and 20 cases were poorly differentiated (Grade 3). The stage of epithelial ovarian carcinoma was categorized using the International Federation of Gynecology and Obstetrics (FIGO) standards, 17 patients were stage I, 8 patients were stage II, 17 patients were stage III, 8 patients were stage IV. 23 of the 50 patients had lymph node metastasis. The mean age of the patients was 55.3 (range 26-78).

CD44 protein expression and its associations with clinicopathological features in epithelial ovarian carcinoma

CD44 immunostaining was observed in the membrane of the epithelial ovarian cancer cells, the representative of immunohistochemical staining are shown in **Figure 1**. CD44 immunoreactivity was detected in 32 tissue sections (64%), but not detected in normal ovarian epithelial tissue. As shown in **Table 2**, there was a significant difference of CD44 expression between normal ovary and ovarian cancer (*P<0.05*). This result verified that the level of

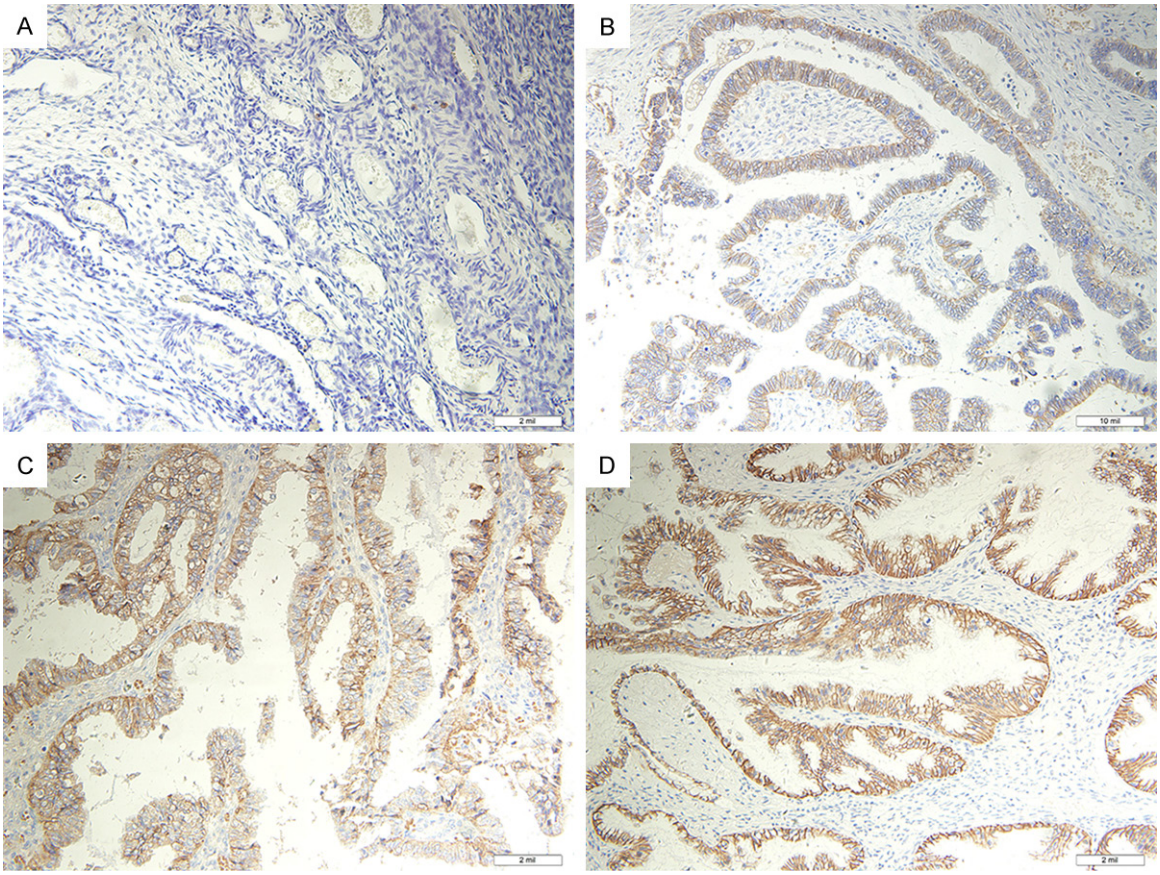

Figure 1. Representative immunohistochemical staining of CD44 in human epithelial ovarian carcinoma and normal ovary tissue specimens. A. Little staining of CD44 in normal ovary. B. Weak expression (+) of CD44 in epithelial ovarian carcinoma tissue. C. Moderate expression (++) of CD44 in epithelia ovarian carcinoma tissue. D. Strong expression (+++) of CD44 in epithelia ovarian carcinoma tissue.

Table 2. Fisher's exact test analyses of CD44 expression in epithelial ovarian cancers and normal ovaries

	n	CD44 expression					*P-value*
		-	+	++	+++	Positive rate (%)	
EOC	50	18	9	13	10	64	<0.001
Normal	15	15	0	0	0	0	

Notes: EOC: epithelia ovarian cancer.

CD44 protein expression was closely associated with epithelial ovarian cancer.

As shown in **Table 1**, the immunohistochemical expression of CD44 in epithelial ovarian cancer significantly correlated with FIGO stage and tumor differentiation (histological grade), lymph node metastasis (*P<0.05*). No significant association was found between CD44 expression and age, histological type in 50 epithelial ovarian cancer patients (*P>0.05*). CD44 expression was higher in patients with FIGO advanced stages (III-IV) and poorly differentiated (G3) than in those with FIGO early stages (I-II) and moderately differentiated and well-differentiated (G1-G2). The percent positive rate of CD44 expression in patients with lymph node metastasis was higher than in those without lymph node metastasis.

EpCAM protein expression and its associations with clinicopathological features in epithelial ovarian carcinoma

Forty cases (80%) of epithelial ovarian carcinoma showed immunoreactivity for EpCAM protein, but only 4 cases (26.7%) of normal ovarian epithelial tissue showed immunoreactivity for EpCAM protein. As shown in **Figure 2**, the typical EpCAM staining was localized in cell membrane. In fact, expression of EpCAM was significantly increased in the epithelial ovarian ca-

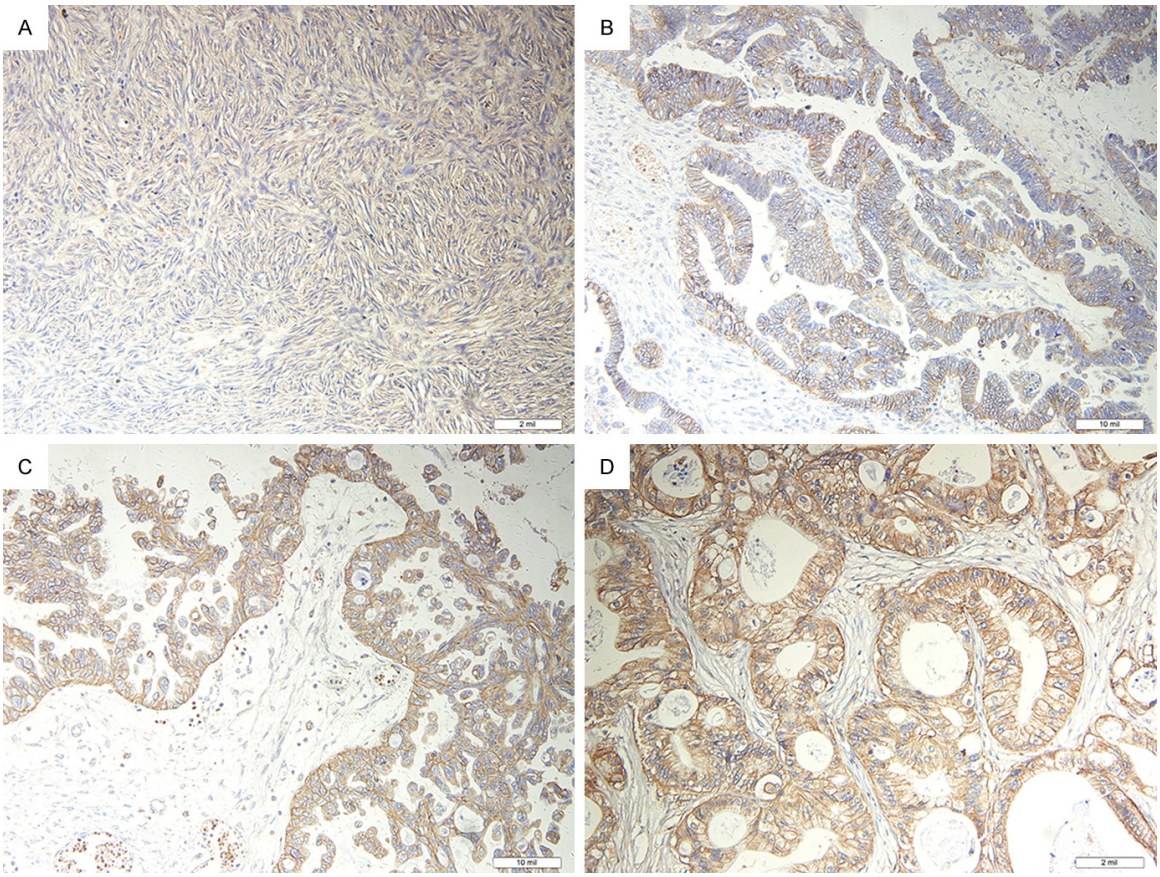

Figure 2. Representative immunohistochemical staining of EpCAM in human epithelial ovarian carcinoma and normal ovary tissue specimens. A. Mild staining of EpCMA in normal ovary tissue. B. Weak expression (+) of EpCAM in epithelial ovarian carcinoma tissue. C. Moderate expression (++) of EpCAM in epithelia ovarian carcinoma tissue. D. Strong expression (+++) of EpCAM in epithelia ovarian carcinoma tissue.

Table 3. Chi-square test analyses of EpCAM expression in epithelia ovarian cancers and normal ovaries

	n	EpCAM expression					X^2	*P-value*
		-	+	++	+++	Positive rate (%)		
EOC	50	10	12	14	14	80	12.668	<0.001
Normal	15	11	2	2	0	26.7		

Table 4. Association between EpCAM and CD44 expression in epithelial ovarian carcinoma

CD44 expression	EpCAM expression		Total	*r*	*P*
	Positive	Negative			
Positive	29	3	32	0.354	0.012
Negative	11	7	18		
Total	40	10	50		

rcinoma when compared with normal ovary (X^2=*12.668, P<0.05,* **Table 3**).

Furthermore, we assessed the correlation between EpCAM expression and clinicopathological characteristics. The data are summarized in **Table 1**. There was a positive correlation between EpCAM expression and FIGO stage and tumor differentiation (histological grade), lymph node metastasis (*P< 0.05*). However, EpCAM expression exhibited no relationship to age, histological type in 50 epithelial ovarian cancer patients (*P>0.05*). The percentage of cases with EpCAM expression is higher in lymph node metastasis patients than in those without lymph node metastasis. Increased EpCAM protein expression was more frequently observed in patients with FIGO advanced stages (III-IV) and poorly differentiated (G3) compared to those with FIGO early stages (I-II) and moderately differentiated and well-differentiated (G1-G2).

Correlated protein expression of EpCAM and CD44 in epithelial ovarian carcinoma

The aforementioned analysis indicated a similar pattern of EpCAM and CD44 immunostaining intensity, we sought to detect if EpCAM and CD44 immunostaining might be correlate to each other. In 40 epithelial ovarian cancer samples with EpCAM immnostaining, 29 (29/40=72.5%) samples exhibited a positive CD44 expression. In 10 epithelial ovarian cancer samples with negative EpCAM expression, 7 (7/10=70%) samples exhibited a negative CD44 expression. EpCAM protein level positively correlated with CD44 protein level, demonstrating a significantly positive association between the two molecules (**Table 4**, *r=0.354, P=0.012*).

Discussion

CD44, as a type of cell-surface adhesion molecules, mediates multiple pathological and physiological processes, including malignancy development, cell adhesion, angiogenesis, wound healing and inflammation. Studies have suggested that CD44 is overexpression and promotes cells migration and metastasis for human solid tumors, including breast carcinoma and ovarian carcinoma [12, 13]. Several studies have shown that the overexpression of CD44 enhance the capacity of proliferation and carcinogenesis in renal cancer and gastric cancer [14, 15]. Up to date, the relationship between CD44 expression and clinical significance in epithelial ovarian cancer remains controversial. The present study suggested that the levels of CD44 expression were increased in epithelial ovarian cancer tissues compared with normal ovarian epithelial tissues, and increased of CD44 expression was significantly associated with FIGO stage and tumor differentiation, lymph node metastasis. The results show that CD44 expression may be involved in disease pathogenesis.

It has been reported that EpCAM was activated via proteolysis, and the activated EpCAM as a mitogenic signal transducer mediated the cell proliferative [16]. EpCAM, as a carcinoma-associated antigen, exerts carcinogenesis via upregulating the proto-oncogene c-myc and the cell cycle regulating genes cyclin A and E, which affect the cell cycle progression and enhance cell proliferation and metabolism. In addition, it has been shown that EpCAM also promotes cell proliferation and tumorigenesis by participating in the nuclear Wnt signaling pathway [17]. EpCAM expression was reported to be correlated with tumor differentiation, stage of disease and metastasis in several human carcinomas, including gastric cancer, breast cancer and so on [18, 19]. Furthermore, it was reported that EpCAM was highly overexpressed in primary, recurrent, and metastatic epithelial ovarian cancer specimens [20]. The current study showed the level of EpCAM expression in epithelial ovarian cancer was higher than in normal ovary, and identified a significantly positive association between EpCAM overexpression and FIGO advanced stages, poor differentiation and lymph node metastasis.

In summary, the present study demonstrated that CD44 and EpCAM expression were closely associated with the occurrence, development, invasion and metastasis of human epithelial ovarian cancer, and there was a positive correlation between CD44 and EpCAM expression. Joint detection of CD44 and EpCAM is conductive to a comprehensive judgment of the malignance degree and metastatic potential of epithelial ovarian cancer. Our results further indicated that CD44-EpCAM-targeted therapy might be a potential strategy in epithelial ovarian cancer.

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Disclosure of conflict of interest

None.

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References

[1] Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J, Jemal A. Global cancer statistics, 2012. CA Cancer J Clin 2015; 65: 87-108.

[2] Siegel RL, Miller KD, Jemal A. Cancer statistics, 2015. CA Cancer J Clin 2015; 65: 5-29.
[3] Williams K, Motiani K, Giridhar PV, Kasper S. CD44 integrates signaling in normal stem cell, cancer stem cell and (pre)metastatic niches. Exp Biol Med (Maywood) 2013; 238: 324-338.
[4] Zoller M. CD44: can a cancer-initiating cell profit from an abundantly expressed molecule? Nat Rev Cancer 2011; 11: 254-267.
[5] Orian-Rousseau V, Ponta H. Perspectives of CD44 targeting therapies. Arch Toxicol 2015; 89: 3-14.
[6] Wang SJ, Bourguignon LY. Role of hyaluronan-mediated CD44 signaling in head and neck squamous cell carcinoma progression and chemoresistance. Am J Pathol 2011; 178: 956-963.
[7] Hoofd C, Wang X, Lam S, Jenkins C, Wood B, Giambra V, Weng AP. CD44 promotes chemoresistance in T-ALL by increased drug efflux. Exp Hematol 2016; 44: 166-171, e117.
[8] van der Gun BT, Melchers LJ, Ruiters MH, de Leij LF, McLaughlin PM, Rots MG. EpCAM in carcinogenesis: the good, the bad or the ugly. Carcinogenesis 2010; 31: 1913-1921.
[9] Imrich S, Hachmeister M, Gires O. EpCAM and its potential role in tumor-initiating cells. Cell Adh Migr 2012; 6: 30-38.
[10] Patriarca C, Macchi RM, Marschner AK and Mellstedt H. Epithelial cell adhesion molecule expression (CD326) in cancer: a short review. Cancer Treat Rev 2012; 38: 68-75.
[11] Munz M, Baeuerle PA, Gires O. The emerging role of EpCAM in cancer and stem cell signaling. Cancer Res 2009; 69: 5627-5629.
[12] Anand MT, Kumar S. CD44: a key player in breast cancer. Indian J Cancer 2014; 51: 247-250.
[13] Ween MP, Oehler MK, Ricciardelli C. Role of versican, hyaluronan and CD44 in ovarian cancer metastasis. Int J Mol Sci 2011; 12: 1009-1029.
[14] Lim SD, Young AN, Paner GP, Amin MB. Prognostic role of CD44 cell adhesion molecule expression in primary and metastatic renal cell carcinoma: a clinicopathologic study of 125 cases. Virchows Arch 2008; 452: 49-55.
[15] Jang BI, Li Y, Graham DY, Cen P. The role of CD44 in the pathogenesis, diagnosis, and therapy of gastric cancer. Gut Liver 2011; 5: 397-405.
[16] Schnell U, Kuipers J, Giepmans BN. EpCAM proteolysis: new fragments with distinct functions? Biosci Rep 2013; 33: e00030.
[17] Schnell U, Cirulli V, Giepmans BN. EpCAM: structure and function in health and disease. Biochim Biophys Acta 2013; 1828: 1989-2001.
[18] Wenqi D, Li W, Shanshan C, Bei C, Yafei Z, Feihu B, Jie L, Daiming F. EpCAM is overexpressed in gastric cancer and its downregulation suppresses proliferation of gastric cancer. J Cancer Res Clin Oncol 2009; 135: 1277-1285.
[19] Abd El-Maqsoud NM, Abd El-Rehim DM. Clinicopathologic implications of EpCAM and Sox2 expression in breast cancer. Clin Breast Cancer 2014; 14: e1-9.
[20] Bellone S, Siegel ER, Cocco E, Cargnelutti M, Silasi DA, Azodi M, Schwartz PE, Rutherford TJ, Pecorelli S, Santin AD. Overexpression of epithelial cell adhesion molecule in primary, metastatic, and recurrent/chemotherapy-resistant epithelial ovarian cancer: implications for epithelial cell adhesion molecule-specific immunotherapy. Int J Gynecol Cancer 2009; 19: 860-866.