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Original Article

Primary peritoneal serous carcinoma: a clinicopathological and immunohistochemical study of six cases

Teng Hou[1], Dongxia Liang[1], Jiehua He[1], Xu Chen[2], Yanna Zhang[1]

[1]*State Key Laboratory of Oncology in South China, Sun Yat-sen University Cancer Center, Guangzhou, GD 510060, China;* [2]*Department of Urology, The First Affiliated Hospital Sun Yat-Sen University, 58 Zhongshan 2nd Road, Guangzhou, GD 510080, China*

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Abstract: Aims: Primary peritoneal serous carcinoma (PPSC) is an unusual neoplasm that has not been properly characterized. To better define the clinicopathological and immunohistochemical features of PPSC, we present 6 such cases. Methods: The 6 patients consisted of one man and 5 women, ranging in age from 45 and 75 years. None of the patients had any history or clinical evidence of tumor elsewhere. The immunohistochemical profile was examined using antibodies against β-catenin, E-cadherin, wnt5a, EGFR, VEGF, vimentin, Ki67, and P53. Results: Of all the 6 PPSC cases, 5 cases presented stage IIIC and 1 case presented stage IV. Microscopically, 5 cases were poorly differentiated and 1 was moderately differentiated. All cases showed positive staining for β-catenin, E-cadherin, vimentin, VEGF, P53, and Ki67, 4 cases expressed EGFR, and all cases were consistently negative for wnt5a. Conclusions: We described 6 cases of PPSC with clinicopathological and immunohistochemical features. The findings provide basic knowledge of PPSC.

Keywords: Primary peritoneal serous carcinoma, epithelial ovarian cancer, serous carcinoma

Introduction

Primary peritoneal serous carcinoma (PPSC) is a rare primary malignancy of the peritoneum. Clinically and histopathologically, PPSC is similar to serous ovarian papillary carcinoma, and most scientist are applying the FIGO staging criteria for epithelial ovarian cancer to determine the stage of PPSC [1]. Studies on the molecular pathogenesis suggested that HER-2/neu, p53 [2], Wilm's tumor suppressor protein (WT1), estrogen and progesterone receptor may be involved in the tumorigenesis of PPSC [3-5].

In most of the reported studies, the emphasis has been on the clinical characteristics of the tumor. However, only a few studies examined the immunohistochemical profiles of PPSC. We herein present 6 cases of PPSC with an emphasis of immunohistochemical features and evaluated their correlation with clinicopathological characteristics.

Materials and methods

This study has been approved by the Sun Yat-Sen University Ethics Committee. Six cases of PPSC were retrieved from the electronic medical records of the Department of Pathology, Sun Yat-Sen University Cancer Center in a period of 20 years (1991–2011). Diagnosis of PPSC was confirmed by the clinical and histologic characteristics, excluding the presence of mesothelioma, ovarian cancer, and occult fallopian tube cancer. Formalin-fixed, paraffin-embedded tissue blocks were available for review and immunohistochemical studies in each case. Immunohistochemical studies for β-catenin (Cell Signaling Technology, USA; 1:100), Wnt5a (Abnova, Taiwan; 1:200), E-cadherin (Invitrogen, USA; 1:100), VEGF (BioGenex, USA; 1:100), EGFR (Invitrogen, USA; 1:200), vimentin (Invitrogen, USA; 1:200), Ki67 (DAKO, Denmark; 1:100), and P53 (Invitrogen, USA; detects mutant p53, 1:200) were performed with concurrent adequate controls.

Table 1. Summary of clinical features

Case	1	2	3	4	5	6
Sex	F	F	M	F	F	F
Age (Years)	70	53	75	45	59	53
FIGO Stage	IIIC	IIIC	IIIC	IIIC	IV	IIIC
Grade	2	3	3	3	3	3
Affected Organs	Ut, Ov, Co, Om	Ut, Ov, Co	Co, Me	Ut, Ov, Co, Om, Me, Li	Ut, Ov, Co, Om, Me	Ut, Ov, Co, Om, Ap
Follow-up	NK	Alive at 9 mo	Dead at 1 mo	Alive at 8 mo	Alive at 64 mo	Alive at 5 mo

Abbreviations: F, female; M, male; NK, not known. Ut, Uterus; Ov, Ovary; OM, omentum; Me, mesentery; Co, colorectum; Ap, appendix; Li, Liver.

Table 2. Summary of immunohistochemical scores and labeling index

Case	1	2	3	4	5	6
β-catenin	12	8	12	12	4	8
E-cadherin	12	4	12	8	8	12
Wnt5a	0	0	0	0	0	0
EGFR	8	0	8	2	4	0
VEGF	6	8	12	12	6	6
Vimentin	3	6	3	3	3	3
P53%	92.9%	91.3%	52.2%	16.7%	92.1%	90.7%
Ki67%	36.3%	25.9%	7.5%	22.6%	6.2%	57.5%

Clinical follow-up information was obtained from the patients' medical charts.

Immunohistochemistry staining was performed according to standard techniques. All stained slides were separately scored by two pathologists. Both the intensity and percentage of IHC staining were analyzed. The intensity was scored as follows: 0, no staining; 1, weak staining; 2, moderate staining; 3, strong staining; and the percentage of stained cells was scored as: 0 (0 positive cells), 1 (1-10% positive cells), 2 (11–50% of positive cells), 3 (51-80% of positive cells), or 4 (81-100% of positive cells). A final score was defined by multiplying the percentage of positive cells by the intensity [6]. The labeling index for Ki-67 and P53 were represented by the ratio of positive cells in relation to total cells using Image J software. Approximately 2000 nuclei were counted in 5 randomly selected high-power fields (40X) in each specimen.

Results

Clinical features

The main clinical features of all 6 cases are summarized in **Table 1**. The patients were 5 women and 1 man aged 45 to 75 years (mean age 59 years) at first surgery. Of all the 6 PPSC cases, 5 (83.3%) was poorly differentiated (grade 2) and 1 (16.7%) was moderately differentiated (grade 3). Surgical stage was IIIC in 5 (83.3%) cases and IV in the remaining 1 (16.7%) case. The main presenting symptoms were related to mass effect and included abdominal swelling, abdominal pain, and pelvic discomfort. The main affected organs included uterus, ovary, omentum, mesentery, colorectum, appendix, and Liver. None of the patients had a previous history or clinical evidence of tumor elsewhere. Follow-up ranged from 1 to 64 months. Four patients developed recurrence and were all alive at the last follow-up. One patient died of cerebral infarction 1 month after surgery. One patient was lost to follow-up.

Immunohistochemical features

Immunohistochemical features are shown in **Table 2**. All the 6 cases were consistently positive for β-catenin (**Figure 1A-C**) and E-cadherin (**Figure 1D**), showing strong staining intensity in more than 80% of tumor cells. Most cases were positive for membranous (6/6) and cytoplasmic (5/6) β-catenin staining, while only 1 case showed nuclear staining of β-catenin. All the 6 cases were consistently negative for wnt5a (**Figure 1E-F**). EGFR was expressed in 4 of 6 cases. Three cases demonstrated more than 80% of positive cells, and the remaining 1 case showed 51% to 80% of positive cells. Staining intensity was classified as weak and moderate in 2 and 2 cases, respectively (**Figure 2A-B**).

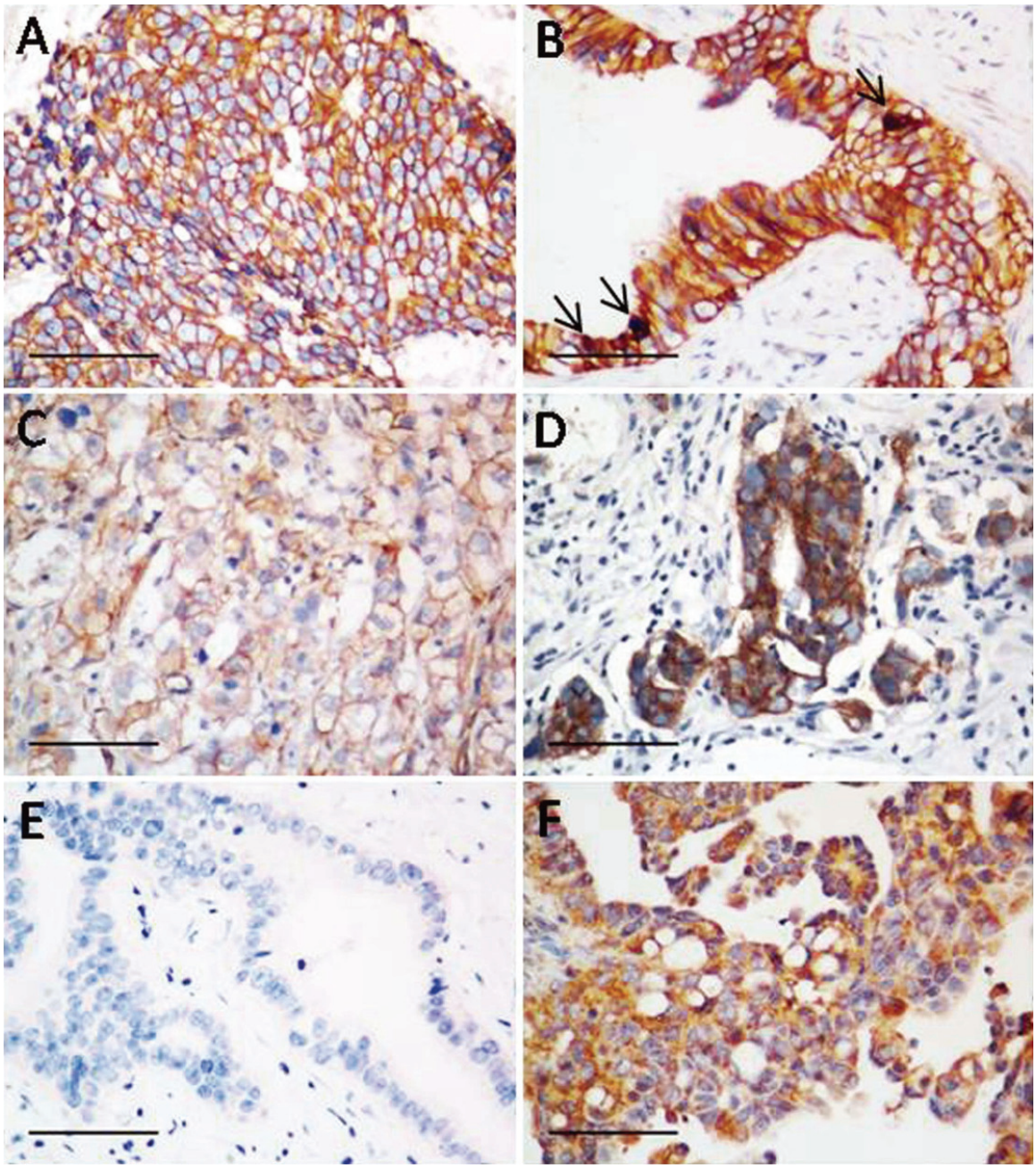

Figure 1. Representative immunostaining of wnt signaling molecules (β-catenin, Wnt5a, and E-cadherin) in PPSC. A: Membranous and cytoplasmic β-catenin staining; B: Membranous β-catenin staining; C: Nuclear β-catenin staining (arrow); D: E-cadherin immunostaining of PPSC; E: wnt5a staining is absent in PPSC; F: wnt5a immunostaining in ovarian cancer as a control. Bars 100 μm.

VEGF was expressed in all cases, with 3 cases demonstrating more than 80% of positive cells, and 3 cases showing 11% to 50% of positive cells. Staining intensity was classified as moderate and strong in 1 and 5 cases, respectively (**Figure 2C**). Vimentin was strongly expressed in all the 6 cases, with 5 cases demonstrating 1% to 10% of positive cells, and the remaining 1 case showing 11% to 50% of positive cells (**Figure 2D**). The immunohistochemical score was defined by multiplying the percentage of positive cells by the intensity to reflect the amount the protein markers expressed by the cancer cells more accurately. Ki-67 and P53

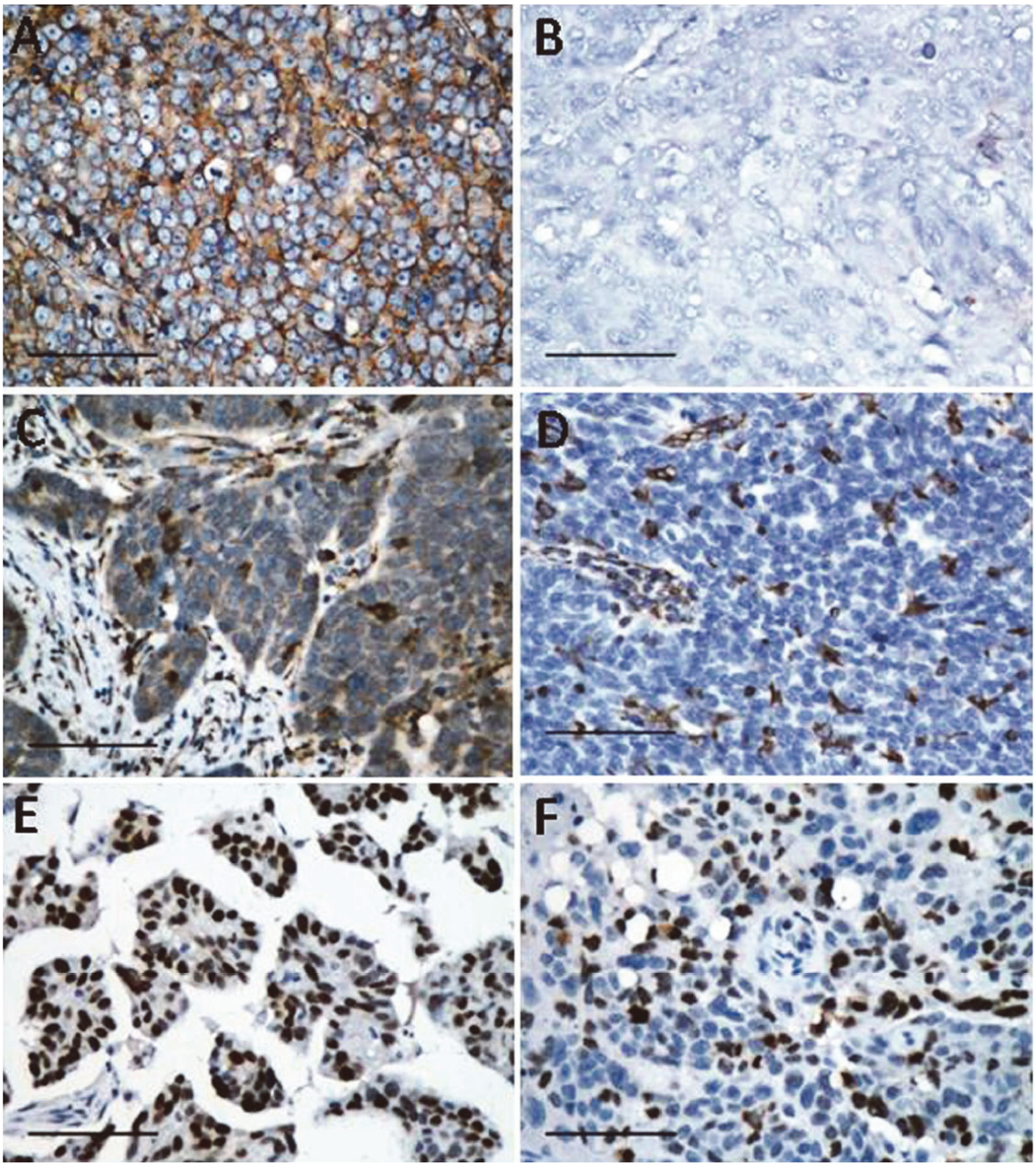

Figure 2. Representative immunostaining of positive EGFR (A), negative EGFR (B) VEGF (C), vimentin (D), mutant P53 (E), and Ki-67 (F) in PPSC. Bars 100μm.

were expressed in all cases, with labeling ranged from 7.5% to 57.5%, and from 16% to 92.9%, respectively (**Figure 2E-F**).

Discussion

The origin of PPSC has not been well characterized. It was thought to arise from the mesothelium of the peritoneum in early studies [7], or from the coelomic epithelium lining the abdominal cavity responding to oncogenic stimulus [8]. More recent data suggested that the fallopian tube may be another source of PPSC [9]. Molecular studies have been inconclusive in illustrating the tumorigenesis of PPSC. Carlson et al. [10] discovered that fimbria is the source of nearly one half of PPSCs by comparing the p53 mutation in both peritoneal and tubal

lesions. Schorge et al. [11] described BRCA1 mutations in 48% of patients with PPSC, of which 89% p53 mutations were observed, which is consistent to our observation that almost all of the PPSC patients express a high level of mutant p53.

There have been many studies demonstrating the clinical and biological similarities between PPSC and epithelial ovarian cancer, as well as some differences. Dubernard et al. [12] compared PPSC and epithelial ovarian cancer in tumor histologic subtype, tumor stage, tumor grade, residual disease, and age. They concluded that the overall survival of patients with PPSC is similar to that of epithelial ovarian cancer group, and that the management of these two diseases should not be different. Choi et al. [13] reported that patients with PPSC have higher levels of CA-125, more omental involvement, and less effect on response to chemotherapy than that with epithelial ovarian cancer. Histologically, the differences of PPSC and epithelial ovarian cancer are currently indistinguishable [4, 14]. Because of the similarity in histological profile, nearly 10% of epithelial ovarian cancer diagnosed were reclassified as PPSC [15]. In addition, the similar histology and close clinical relationship of PPSC and epithelial ovarian cancer indicates that they may develop from the same origin [16].

To date, there have been few comprehensive immunohistochemical studies of PPSC. Von Riedenauer et al. [4] showed positive estrogen receptor (ER), cytokeratin 7 (CK7), Wilm's tumor suppressor gene (WT1), and cancer antigen 125 (CA 125) staining in PPSC. Chen et al. [17], who examined 32 patients of PPSC, showed that the samples were positive for HER-2/neu (34.4%), p53 (71.9%), bcl-2 (9.4%), and nm23-H1 (100%). Barnetson et al. [14], who investigated 14 cases of PPSC, have shown strong expression of Ber-EP4 (86%), Mesothelin (71%), MOC31 (71%), CA 125 (79%), and ER (86%).

Based on the presence of the histologic features of PPSC, we made an attempt at discovering some potential molecular markers that could help understanding the molecular mechanisms of PPSC tumorigenesis. We investigated the expression of key molecules of wnt signaling (β-catenin, Wnt5a, and E-cadherin), which have been proved to be involved in ovarian tumorigenesis [18], in PPSC. As the result, we found that the tumor cells were strong positive for β-catenin and E-cadherin, but consistently negative for Wnt5a. It has been proposed that the nuclear localization of β-catenin was a prognostic marker in a number of human cancers. Verghese et al. [19] described that the fibroblasts with nuclear β-catenin in tumors is a good prognostic indicator for breast cancer. Liu et al. [20] reported that β-catenin was positively correlated with the Karnofsky performance scale (KPS) score and World Health Organization (WHO) grades of human gliomas. Kildal et al. [21] demonstrated that nuclear β-catenin localization was positively correlated associated with good prognostic outcome in patients with ovarian cancer, and that higher nuclear β-catenin expression was observed in grade 1 (16%) and 2 (24%) than in grade 3 (6%) ovarian carcinomas. The present study concurs with those previous studies in that nuclear β-catenin expression was uncommon in high grade tumors.

It has been shown that the expression of E-cadherin was significantly positively correlated with overall survival of ovarian carcinoma, probably through suppressing tumor invasion and metastasis [22, 23]; yet, there have been reports demonstrating that E-cadherin may facilitate the ovarian tumor cells aggregation, adherence and invasion to the peritoneum, which results in coelomic metastasis of ovarian cancer [24, 25]. Our data may explain the correlation of multiple organs involvement in PPSC and the cell migration and invasion-promoting effects of E-cadherin. Moreover, some other studies assessed the correlation of nuclear β-catenin expression and the loss of E-cadherin in tumor invasion [26, 27]. However, no such reverse association between nuclear β-catenin and E-cadherin expression was found in this study. This could be because of the small sample size, or the intrinsic tumor heterogeneity.

Wnt5a was found to be highly expressed in high-grade ovarian carcinoma [28] and several malignancies such as stomach, prostate, melanoma, and breast [29]. It may be useful in predicting the prognosis and chemosensitivity to anticancer drugs in ovarian cancers. Kurayoshi et al. [30] suggested that Wnt-5a is correlated with tumor aggressiveness by stimulating cell migration and invasion. However, wnt5a expression was negative in all the present cases, which implies that wnt5a could represent a

potential new marker to distinguish epithelial ovarian cancer and PPSC.

We also investigated the expression of VEGF, EGFR, vimentin, and P53, which play critical roles in serous carcinoma carcinogenesis. It is well known that VEGF contribute to tumor angiogenesis and progression, and that it promotes ascites accumulation in ovarian cancers [24]. VEGF expression was all positive in the present cases, as expected. The EGFR and vimentin expression was 67% (4/6) and 33% (2/6), respectively. EGFR has been reported to be expressed in ovarian cancers and is associated with poor prognosis [31]. Vimentin is a member of the intermediate filament protein family. In addition, it represents a potential new marker for epithelial-mesenchymal transition (EMT) [32]. The mean P53 labeling index was 82%, suggesting that P53 mutations are frequent in PPSC. The mean Ki-67 labeling index in the present study was 30%, indicating moderate proliferative activity of PPSC. These immunohistochemical data may provide basic knowledge of PPSC.

PPSC predominantly affects postmenopausal women. In our series, five cases also occurred in postmenopausal women, while only one of our patients was a 75-year-old man. Interestingly, the Ki67 labeling index in this male patient was only 7.5%, which was significantly lower than that in female patients. With regards to other markers, there were no significant difference between the male case and the remaining female cases. Further studies are needed to better understand the underlying links between different sexual immunoprofiles and clinical characteristics.

Conclusion

In summary, we have described the clinicopathologic and immunohistochemical findings in 6 patients with PPSC. We studied the molecular changes occurring in PPSC and analyzed their potential roles in tumorigenesis, which may enable the discovery of biomarkers and targeted therapeutic agents. In addition, we found that wnt5a may be useful in the differential diagnosis of PPSC.

Address correspondence to: Dr. Yanna Zhang, State Key Laboratory of Oncology in South China, 651 E Dongfeng Ave, Guangzhou, GD 510060, China. Tel: 8600 18718908165; Fax: 8600 2087343014; E-mail: zhangyn@sysucc.org.cn

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