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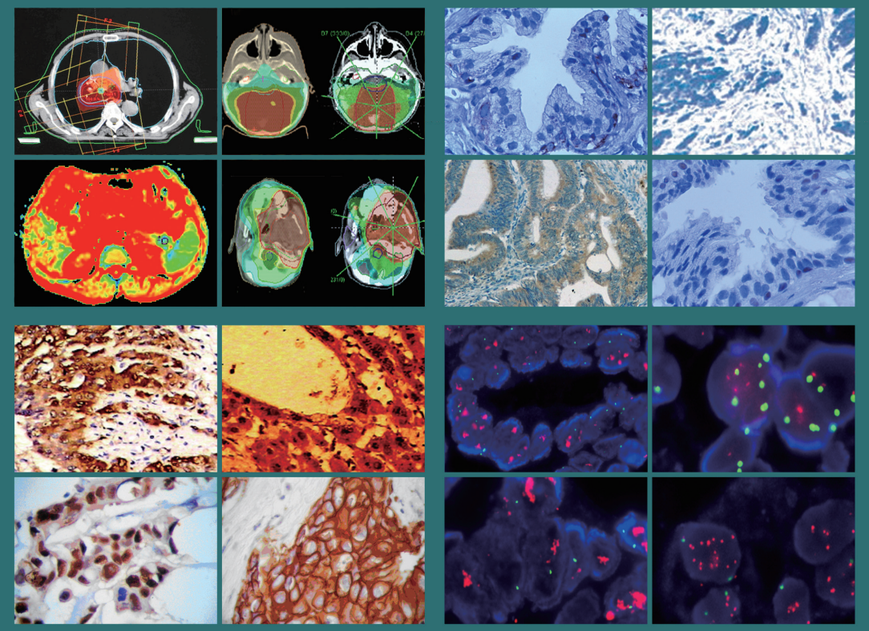

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Aims & Scope

Oncology and Translational Medicine is an international professional academic periodical. The Journal is designed to report progress in research and the latest findings in domestic and international oncology and translational medicine, to facilitate international academic exchanges, and to promote research in oncology and translational medicine as well as levels of service in clinical practice. The entire journal is published in English for a domestic and international readership.

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ORIGINAL ARTICLE

Immunohistochemical panel of glypican-3, hepatocyte paraffin antigen-1, arginase-1, cytokeratin-19, and human epithelial membrane antigen for the differential diagnosis of liver tumors*

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Abstract

Objective Clinical immunohistochemistry plays an increasingly important role in pathologic diagnosis. We investigated the usefulness of an immunohistochemical panel of glypican-3 (GPC3), hepatocyte paraffin antigen-1 (HepPar-1), arginase-1 (Arg-1), cytokeratin-19 (CK19), and human epithelial membrane antigen (EMA) for the differential diagnosis of liver tumors.

Methods Two hundred and thirty-five immunohistochemical sections of hepatocellular carcinoma (HCC; 120 cases), intrahepatic cholangiocarcinoma (ICC; 50 cases), combined hepatocellular and cholangiocarcinoma (CHC; 17 cases), metastatic adenocarcinoma (20 cases), and benign liver lesions (28 cases) were obtained from the Department of Pathology at Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China. The sensitivity and specificity of the combined biomarkers GPC3/HepPar-1/Arg-1/CK19/EMA for the differential diagnosis of HCC, ICC, and CHC were calculated and analyzed retrospectively.

Results The combined biomarkers $GPC3^+/CK19^-$ had the highest specificity (98.3%) for diagnosing HCC, with a sensitivity of 60.0%. The specificity of $GPC3^-/HepPar\text{-}1^-/Arg\text{-}1^-/CK19^+/EMA^+$ for diagnosing ICC was 93.0%, with a sensitivity of 76.0%. The specificity of $GPC3^+/HepPar\text{-}1^+/Arg\text{-}1^+/CK19^+/EMA^+$ for diagnosing CHC was 95.9%, with a sensitivity of 52.9%.

Conclusion The combined biomarkers GPC3/HepPar-1/Arg-1/CK19/EMA greatly improved the specificity of liver tumor diagnosis. We believe that clinical pathological work could improve the original determination of liver nodules.

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Key words: hepatocellular carcinoma (HCC); intrahepatic cholangiocarcinoma (ICC); combined hepatocellular and cholangiocarcinoma (CHC); immunohistochemistry

Hepatocellular carcinoma (HCC) is the third most frequent cause of cancer-related deaths worldwide [1]. The current gold standard for HCC diagnosis is pathological examination; however, some complicated cases can be difficult to determine, such as patients with intrahepatic cholangiocarcinoma (ICC) or combined hepatocellular and cholangiocarcinoma (CHC) [2–3]. Various immunohistochemical biomarkers have played an increasingly important role in assisting pathological diagnosis of cellular origin [4–5].

Glypican-3 (GPC3) is an important member of the glypican family that is attached to the cell membrane via a glycosyl-phosphatidyl-inositol (GPI) anchor. GPC3 was first reported by Hsu *et al* in 1997 [6], with its protein levels in cancerous and normal liver tissues confirmed by a subsequent study [7–9]. GPC3 has a close interaction

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with the Wnt, Yap, and FGF signaling pathways, which are thought to promote HCC formation and progression [10–13]. Hepatocyte paraffin antigen-1 (HepPar-1) is a surface antigen from hepatic mitochondria that is highly expressed in tissues of hepatocytic origin; it has been reported that the sensitivity of HepPar-1 during HCC diagnosis could be as high as 90% [14–16]. Arginase (Arg-1) is an enzyme that can metabolize arginine into urea and ornithine. Like HepPar-1, Arg-1 has adequate diagnostic sensitivity for HCC. Studies have shown that the sensitivity of Arg-1 for HCC detection could be > 90%, higher than alpha-foetoprotein (AFP), GPC3, and HepPar-1 [14–18]. However, both Arg-1 and HepPar-1 are expressed in some benign liver lesions, thus their diagnostic specificity for HCC is inferior to GPC3 and AFP. As for ICC diagnosis, cytokeratin-19 (CK19) is expressed in various single-layer epithelial tissues and exhibits high sensitivity [19–21]. CK19 is mainly used to differentiate adenocarcinoma from HCC in intrahepatic lesions. Human epithelial membrane antigen (EMA) is a specific tumor biomarker since its protein epitope is associated with abnormal glycosylation. As a member of transmembrane glycoprotein family, EMA is expressed in various epithelial tissues, such as ICC [19, 22]; however, both CK19 and EMA exhibit inadequate specificity for ICC since their expression can also be detected in metastatic adenocarcinoma and CHC.

To combine the characteristics of each biomarker, Timek *et al* reported that Arg-1, HepPar-1, and GPC3 formed the most effective biomarker panel for distinguishing HCC from metastatic tumors [23], whilst Ryu *et al* suggested that GPC3 and CK19 could be used as first-line markers for the differential diagnosis of HCC and ICC [20]. In this study, we used a large data set to assess the utility of the GPC3/HepPar-1/Arg-1/CK19/EMA immunohistochemical panel for differentially diagnosing intrahepatic lesions, applicable for HCC, ICC, CHC, metastatic adenocarcinoma, and benign liver lesions. This is the first report to recommend the GPC3/HepPar-1/Arg-1/CK19/EMA panel for the differential diagnosis of liver tumors. We believe that the panel will facilitate pathological diagnosis in a clinical setting.

Materials and methods

Patients and tissue samples

Patients who had been simultaneously tested for the immunohistochemical biomarkers GPC3, HepPar-1, Arg-1, CK19, and EMA were selected from the Department of Pathology of Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China, between January 2012 and May 2015 (120 HCC cases, 50 ICC cases, 17 CHC cases, 20 metastatic adenocarcinoma cases, and 28 benign liver lesion cases). The origins of metastatic liver adenocarcinoma included pancreatic cancer (6 cases), lung cancer (6 cases), gastric cancer (3 cases), colon cancer (2 cases), rectal cancer (2 cases) and breast cancer (1 case). Benign liver lesions included inflammatory liver hyperplasia (7 cases), fibrous liver hyperplasia (9 cases), and focal nodular liver hyperplasia (12 cases). The final decision for all cases was made by macro- and micro-pathological observations, with immunohistochemical tests assisting the diagnosis. The sensitivity and specificity of each biomarker and the combined biomarkers for the diagnosis of liver tumors was calculated. Samples were acquired from the resections or biopsies of HCC, ICC, CHC, metastatic adenocarcinoma, and benign liver lesions. All tissues were routinely fixed in 10% neutral buffered formalin and paraffin, with slides independently reviewed by two pathologists.

Immunohistochemistry and interpretation

Immunohistochemistry was performed according to the standard protocol of the Department of Pathology of Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China. Prior to immunohistochemical staining, all available slides were routinely subjected to hematoxylin and eosin (HE) staining to identify tissue blocks with tumor architecture. The tissue blocks were then fixed in formalin, embedded in paraffin, sectioned at a thickness of 5 μm, deparaffinized, and rehydrated. For antigen retrieval, sections were soaked in 10 mM citrate buffer in a pressure cooker for 3 min. Endogenous peroxidase activity was inhibited by treatment with 3% hydrogen peroxide for 3 min and nonspecific binding sites were blocked with 10% non-immune goat serum for 30 min. The slides were treated with the following individual primary monoclonal antibodies (against GPC3, HepPar-1, Arg-1, CK19, and EMA biomarkers) at 4 °C overnight: mouse anti-GPC3 (1:150), mouse anti-HepPar-1 (1:120), rabbit anti-Arg-1 (1:120), mouse anti-CK19 (1:150), and mouse anti-EMA (1:150). The antibodies were all purchased from ZSGB-BIO (Beijing, China) and an Envision Kit + Dual Link System HRP was used to develop the histological images. Diaminobenzidine (DAB) was used as the chromogen for immunostaining. Phosphate-buffered saline (PBS, pH 7.2) was used instead of primary antibodies as the negative control, and specific GPC3, HepPar-1, Arg-1, CK19, and EMA positive samples confirmed by western blotting were used as positive controls. Before examination under an Olympus microscopic digital camera, all slides were counterstained with hematoxylin.

To interpret the immunohistochemistry results, two pathologists independently analyzed the staining under a microscope. GPC3, HepPar-1, Arg-1, CK19, and EMA were located in the cytoplasm and membrane, cytoplasm, cytoplasm and nuclei, cytoplasm, and cytoplasm,

respectively. Histological scores were given by two methods: (1) Scoring by staining intensity (0: no signal; 1: weak; 2: moderate; and 3: marked); (2) Scoring by the percentage of immunoreactive cells (0: 0%; 1: ≤ 10%; 2: > 10%–50%; 3: 50%–75%; 4: > 75%). After multiplying the two scores, scores of > 3 was considered positive expression and scores of < 3 were considered negative expression.

Statistical analysis

Calculations were performed using the SPSS 17.0 software package. Differences between the rates of biomarker positivity in different liver lesions were analyzed using chi-square tests. Values of $P < 0.05$ were considered significant.

Results

This retrospective study analyzed the ability of the GPC3/HepPar-1/Arg-1/CK19/EMA panel of immunohistochemical biomarkers to differentially diagnose liver tumors. The single biomarkers with the highest sensitivity and specificity for HCC diagnosis were Arg-1 (90.0%) and GPC3 (79.1%), respectively (Table 1). The single biomarkers with the highest sensitivity and specificity for ICC diagnosis were CK19 (98.0%) and EMA (64.9%), respectively (Table 2). The combined biomarkers $GPC3^+/CK19^-$ had the highest specificity (98.3%) for HCC diagnosis, with a sensitivity of 60.0% (Table 3). The diagnostic efficiency of $GPC3^+/EMA^-$ (specificity: 97.4%; sensitivity: 60.8%) for HCC was very similar to that of $GPC3^+/CK19^-$. The specificity of $GPC3^-/HepPar\text{-}1^-/Arg\text{-}1^-/CK19^+/EMA^+$ for ICC diagnosis was 93.0%, with a sensitivity of 76.0% (Table 4). The specificity of $GPC3^+/HepPar\text{-}1^+/Arg\text{-}1^+/CK19^+/EMA^+$ for CHC diagnosis was 95.9%, with a sensitivity of 52.9% (Table 5).

The staining sites of GPC3, HepPar-1, Arg-1, CK19, and EMA were the cytoplasm and membrane, cytoplasm, cytoplasm and nuclei, cytoplasm, and cytoplasm, respectively. The representative staining pattern of the immunohistochemical biomarkers in different liver tumors was as follows: 1. GPC3, HepPar-1, and Arg-1 were highly expressed in HCC, whilst CK19 and EMA were almost unidentifiable (Fig. 1); 2. CK19 and EMA were highly expressed in ICC whilst GPC3, HepPar-1 and Arg-1 expression was relatively low (Fig. 2), with the staining features almost the same in metastatic adenocarcinoma (Fig. 3); 3. GPC3, HepPar-1, Arg-1, CK19, and EMA were all highly expressed in CHC (Fig. 4); and 4. HepPar-1 and Arg-1 were positively expressed in benign liver lesions, whilst GPC3, CK19, and EMA were barely detectable (Fig. 5).

Discussion

Liver tumors pose a serious problem for human health, with accurate diagnosis and early intervention critical for extending survival time and improving quality of life [24–26]. However, for some complicated clinical cases it can be difficult to differentiate between liver tumors; thus, treatment can be seriously hampered due to inaccurate or delayed diagnosis. AFP is a traditional biomarker for HCC diagnosis, with AFP levels being an important indicator for HCC [14, 27–28]; however, a large number of HCC patients are AFP-negative [14, 28]. It has been reported that AFP immunoreactivity was only detected in 40 of 78 (51.3%) HCC cases by immunohistochemistry [14]. Multiple biomarkers could improve the determination of disease origin and reduce the rate of misdiagnosis and

Table 1 Expression level of GPC3, HepPar-1, and Arg-1 in different liver lesions

	HCC (n = 120)	ICC (n = 50)	P^1	CHC (n = 17)	P^2	Metastatic adenocarcinoma (n = 20)	P^3	Benign liver lesions (n = 28)	P^4	Sensitivity (%)	Specificity (%)
$GPC3^+$	95	6	0.000	14	0.760	2	0.000	2	0.000	79.2	79.1
$HepPar\text{-}1^+$	96	3	0.000	12	0.374	3	0.000	23	0.797	80.0	64.3
$Arg\text{-}1^+$	108	4	0.000	14	0.345	0	NA	25	0.910	90.0	62.6

P^1, P^2, P^3 and P^4 represented difference comparison of biomarkers' positive rates between HCC and ICC, CHC, metastatic adenocarcinoma, benign liver lesions respectively. NA: Not available. The sample cases were 0, and process can't be conducted.

Table 2 Expression level of CK19 and EMA in different liver lesions

	ICC (n = 50)	HCC (n = 120)	P^1	CHC (n = 17)	P^2	Metastatic adenocarcinoma (n = 20)	P^3	Benign liver lesions (n = 28)	P^4	Sensitivity (%)	Specificity (%)
$CK19^+$	49	26	0.000	17	NA	19	0.496	8	0.000	98.0	62.2
EMA^+	48	27	0.000	16	0.746	18	0.329	4	0.000	96.0	64.9

P^1, P^2, P^3 and P^4 represented difference comparison of biomarkers' positive rates between ICC and HCC, CHC, metastatic adenocarcinoma, benign liver lesions respectively. NA: Not available. The sample cases were 0, and process can't be conducted.

Table 3 Combined biomarkers for diagnosis of HCC (*n*, %)

	HCC (*n* = 120)	ICC (*n* = 50)	CHC (*n* = 17)	Metastatic adenocarcinoma (*n* = 20)	Benign liver lesions (*n* = 28)	Sensitivity (%)	Specificity (%)
Two							
$GPC3^+/HepPar\text{-}1^+$	76	2	10	0	2	63.3	87.8
$GPC3^+/Arg\text{-}1^+$	86	2	12	0	2	71.7	86.1
$HepPar\text{-}1^+/Arg\text{-}1^+$	93	1	12	0	21	80.9	70.4
$GPC3^+/CK19^-$	72	0	0	0	2	60.0	98.3
$HepPar\text{-}1^+/CK19^-$	79	0	0	0	18	65.8	84.3
$Arg\text{-}1^+/CK19^-$	86	0	0	0	18	71.7	84.3
$GPC3^+/EMA^-$	73	0	1	0	2	60.8	97.4
$HepPar\text{-}1^+/EMA^-$	77	0	1	0	22	64.2	80.0
$Arg\text{-}1^+/EMA^-$	87	0	1	0	22	72.5	80.0
Three							
$GPC3^+/HepPar\text{-}1^+/Arg\text{-}1^+$	76	1	10	0	2	63.3	88.7
$GPC3^+/HepPar\text{-}1^+/CK19^-$	62	0	0	0	2	51.7	98.3
$GPC3^+/Arg\text{-}1^+/CK19^-$	67	0	0	0	2	55.8	98.3
$HepPar\text{-}1^+/Arg\text{-}1^+/CK19^-$	77	0	0	0	16	64.2	86.1
$GPC3^+/HepPar\text{-}1^+/EMA^-$	59	0	1	0	2	49.2	97.4
$GPC3^+/Arg\text{-}1^+/EMA^-$	68	0	1	0	2	56.7	97.4
$HepPar\text{-}1^+/Arg\text{-}1^+/EMA^-$	76	0	1	0	20	63.3	81.7
$GPC3^+/CK19^-/EMA^-$	60	0	0	0	2	50.0	98.3
$HepPar\text{-}1^+/CK19^-/EMA^-$	69	0	0	0	17	57.5	85.2
$Arg\text{-}1^+/CK19^-/EMA^-$	75	0	0	0	18	62.5	84.3
Four							
$GPC3^+/HepPar\text{-}1^+/CK19^-/EMA^-$	52	0	0	0	2	43.3	98.3
$GPC3^+/Arg\text{-}1^+/CK19^-/EMA^-$	57	0	0	0	2	47.5	98.3
$HepPar\text{-}1^+/Arg\text{-}1^+/CK19^-/EMA^-$	67	0	0	0	16	55.8	86.1
$GPC3^+/HepPar\text{-}1^+/Arg\text{-}1^+/CK19^-$	60	0	0	0	2	50.0	98.3
$GPC3^+/HepPar\text{-}1^+/Arg\text{-}1^+/EMA^-$	56	0	1	0	2	46.7	97.4
Five							
$GPC3^+/HepPar\text{-}1^+/Arg\text{-}1^+/CK19^-/EMA^-$	51	0	0	0	2	42.5	98.3

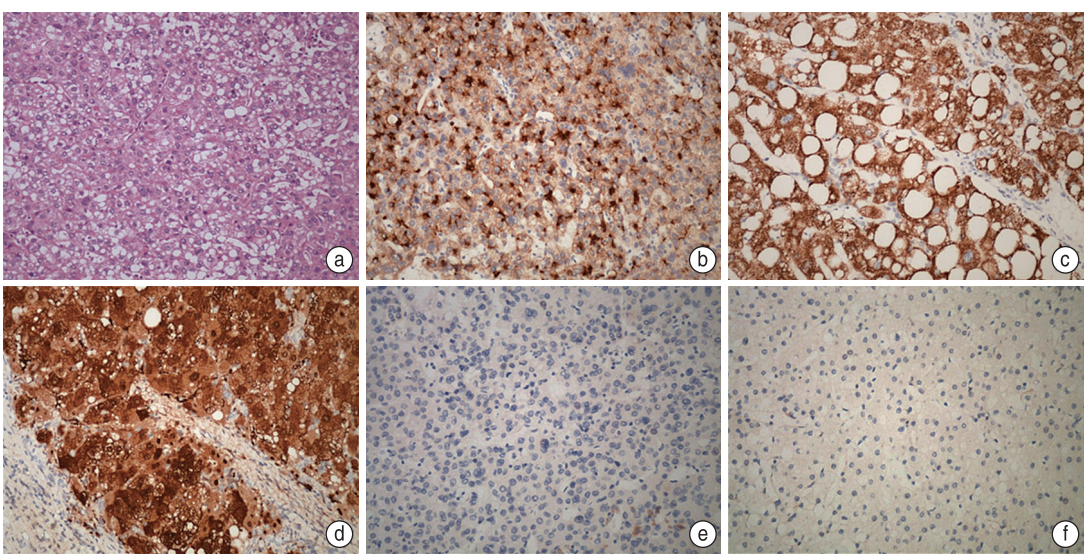

Fig. 1 HE staining of HCC and expression level of different biomarkers in HCC. (a) HE staining of HCC. (b) GPC3 was highly expressed in HCC, and the staining sites were cytoplasm and membrane. (c) HepPar-1 was highly expressed in HCC, and the staining site was cytoplasm. (d) Arg-1 was highly expressed in HCC, and the staining sites were cytoplasm and nuclei. CK19 (e) and EMA (f) were almost not presented in HCC. (× 200)

Table 4 Combined biomarkers for diagnosis of ICC (*n*, %)

	ICC (*n* = 50)	HCC (*n* = 120)	CHC (*n* = 17)	Metastatic adenocarcinoma (*n* = 20)	Benign liver lesions (*n* = 28)	Sensitivity (%)	Specificity (%)
Two							
$CK19^+/EMA^+$	47	12	16	17	4	94.0	73.5
$GPC3^-/CK19^+$	43	3	3	18	8	86.0	82.7
$HepPar\text{-}1^-/CK19^+$	46	4	5	16	3	92.0	84.9
$Arg\text{-}1^-/CK19^+$	45	4	3	19	1	90.0	85.4
$GPC3^-/EMA^+$	42	5	3	16	4	84.0	84.9
$HepPar\text{-}1^-/EMA^+$	45	8	5	15	3	90.0	85.2
$Arg\text{-}1^-/EMA^+$	44	6	3	18	1	88.0	84.9
Three							
$GPC3^-/HepPar\text{-}1^-/CK19^+$	42	1	1	14	3	84.0	89.7
$GPC3^-/Arg\text{-}1^-/CK19^+$	41	0	1	17	0	82.0	90.3
$HepPar\text{-}1^-/Arg\text{-}1^-/CK19^+$	43	4	3	16	0	86.0	87.6
$GPC3^-/HepPar\text{-}1^-/EMA^+$	41	3	1	14	3	82.0	88.6
$GPC3^-/Arg\text{-}1^-/EMA^+$	40	2	1	16	1	80.0	89.2
$HepPar\text{-}1^-/Arg\text{-}1^-/EMA^+$	42	5	3	15	1	84.0	87.0
$GPC3^-/CK19^+/EMA^+$	41	2	3	15	4	82.0	87.0
$HepPar\text{-}1^-/CK19^+/EMA^+$	45	3	5	14	4	90.0	85.9
$Arg\text{-}1^-/CK19^+/EMA^+$	43	2	3	17	1	86.0	87.6
Four							
$GPC3^-/HepPar\text{-}1^-/CK19^+/EMA^+$	41	1	1	12	3	82.0	90.8
$GPC3^-/Arg\text{-}1^-/CK19^+/EMA^+$	40	0	1	15	0	80.0	91.4
$HepPar\text{-}1^-/Arg\text{-}1^-/CK19^+/EMA^+$	41	2	3	14	0	82.0	89.7
$GPC3^-/HepPar\text{-}1^-/Arg\text{-}1^-/CK19^+$	40	0	1	14	0	80.0	91.9
$GPC3^-/HepPar\text{-}1^-/Arg\text{-}1^-/EMA^+$	39	1	1	13	1	78.0	91.4
Five							
$GPC3^-/HepPar\text{-}1^-/Arg\text{-}1^-/CK19^+/EMA^+$	38	0	1	12	0	76.0	93.0

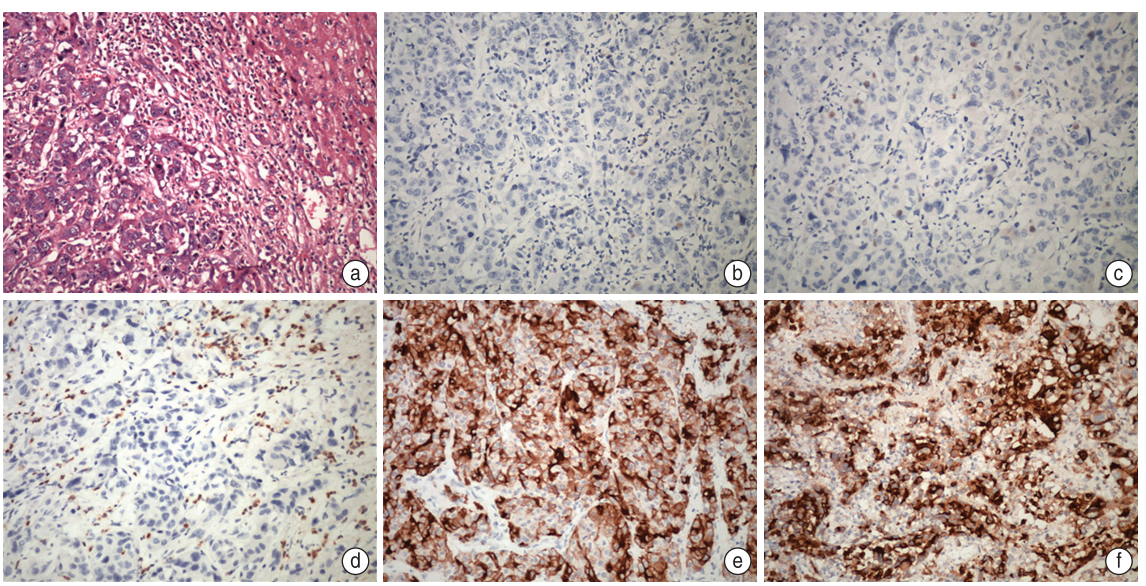

Fig. 2 HE staining of ICC and expression level of different biomarkers in ICC. (a) HE staining of ICC. GPC3 (b), HepPar-1 (c) and Arg-1 (d) were negatively expressed in ICC. CK19 (e) and EMA (f) were highly expressed in ICC, and the staining site was located at cytoplasm. (× 200)

Table 5 Combined biomarkers for diagnosis of CHC (*n*, %)

	CHC (*n* = 17)	HCC (*n* = 120)	ICC (*n* = 50)	Metastatic adenocarcinoma (*n* = 20)	Benign liver lesions (*n* = 28)	Sensitivity (%)	Specificity (%)
Two							
GPC3$^+$/CK19$^+$	14	23	6	2	0	82.4	85.8
HepPar-1$^+$/CK19$^+$	12	16	3	3	5	70.6	87.6
Arg-1$^+$/CK19$^+$	14	21	4	0	7	82.4	85.3
GPC3$^+$/EMA$^+$	13	22	6	2	0	76.5	86.2
HepPar-1$^+$/EMA$^+$	11	19	3	3	1	64.7	88.1
Arg-1$^+$/EMA$^+$	13	21	4	0	3	76.5	87.2
Three							
GPC3$^+$/HepPar-1$^+$/CK19$^+$	10	15	2	0	0	58.8	92.2
GPC3$^+$/Arg-1$^+$/CK19$^+$	12	19	2	0	0	70.6	90.4
HepPar-1$^+$/Arg-1$^+$/CK19$^+$	12	17	1	0	5	70.6	89.4
GPC3$^+$/HepPar-1$^+$/EMA$^+$	9	17	2	0	0	52.9	91.3
GPC3$^+$/Arg-1$^+$/ EMA$^+$	11	20	2	0	0	64.7	89.9
HepPar-1$^+$/Arg-1$^+$/EMA$^+$	11	18	1	0	1	64.7	90.8
GPC3$^+$/CK19$^+$/EMA$^+$	13	10	6	2	0	76.5	91.7
HepPar-1$^+$/CK19$^+$/EMA$^+$	11	9	3	3	1	64.7	92.7
Arg-1$^+$/CK19$^+$/EMA$^+$	13	10	3	0	2	76.5	93.1
Four							
GPC3$^+$/HepPar-1$^+$/CK19$^+$/EMA$^+$	9	8	2	0	0	52.9	95.4
GPC3$^+$/ Arg-1$^+$/CK19$^+$/EMA$^+$	11	8	2	0	0	64.7	95.4
Hepar-1$^+$/Arg1$^+$/CK19$^+$/EMA$^+$	11	9	1	0	1	64.7	95.0
GPC3$^+$/HepPar-1$^+$/Arg-1$^+$/CK19$^+$	10	13	1	0	0	58.8	93.6
GPC3$^+$/HepPar-1$^+$/Arg-1$^+$/EMA$^+$	9	16	1	0	0	52.9	92.2
Five							
GPC3$^+$/HepPar-1$^+$/Arg-1$^+$/CK19$^+$/EMA$^+$	9	8	1	0	0	52.9	95.9

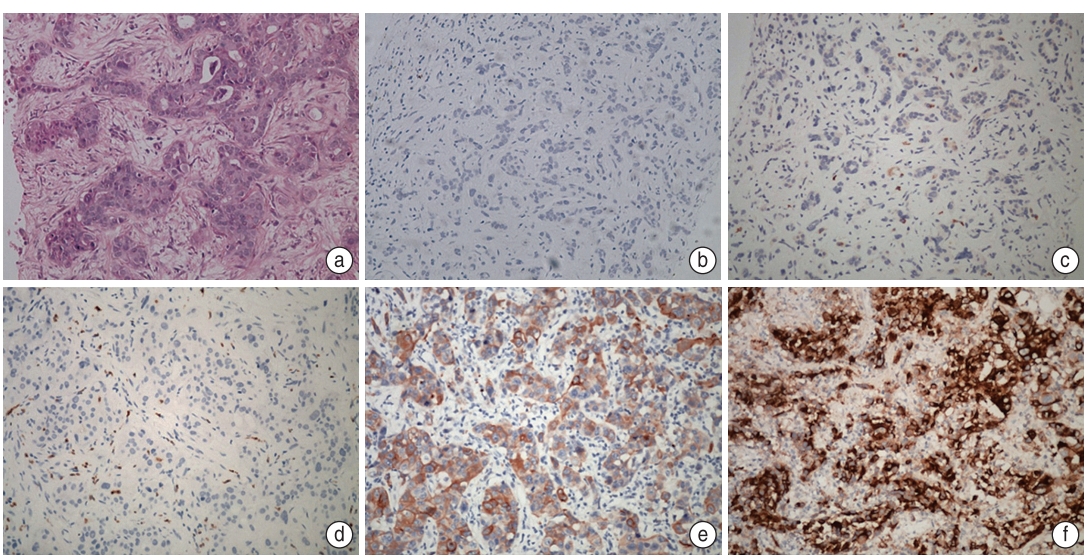

Fig. 3 HE staining of metastatic adenocarcinoma and expression level of different biomarkers in metastatic adenocarcinoma. (a) HE staining of metastatic adenocarcinoma. GPC3 (b), HepPar-1 (c) and Arg-1 (d) were negatively expressed in metastatic adenocarcinoma, while CK19 (e) and EMA (f) were highly expressed. (× 200)

missed diagnosis.

This study analyzed the usefulness of an GPC3/HepPar-1/Arg-1/CK19/EMA immunostaining panel for diagnosing and differentially diagnosing liver tumors. GPC3 is a cell surface proteoglycan that is highly expressed in early HCC but little expressed in benign

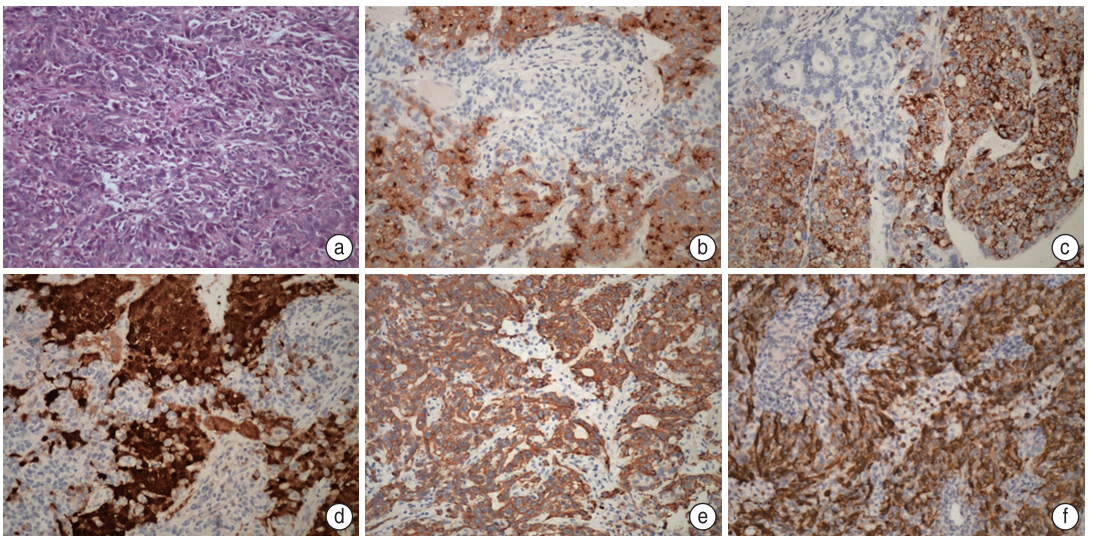

Fig. 4 HE staining of CHC and expression level of different biomarkers in CHC. (a) HE staining of CHC. GPC3 (b), HepPar-1 (c), Arg-1 (d), CK19 (e) and EMA (f) were all highly expressed in CHC. (× 200)

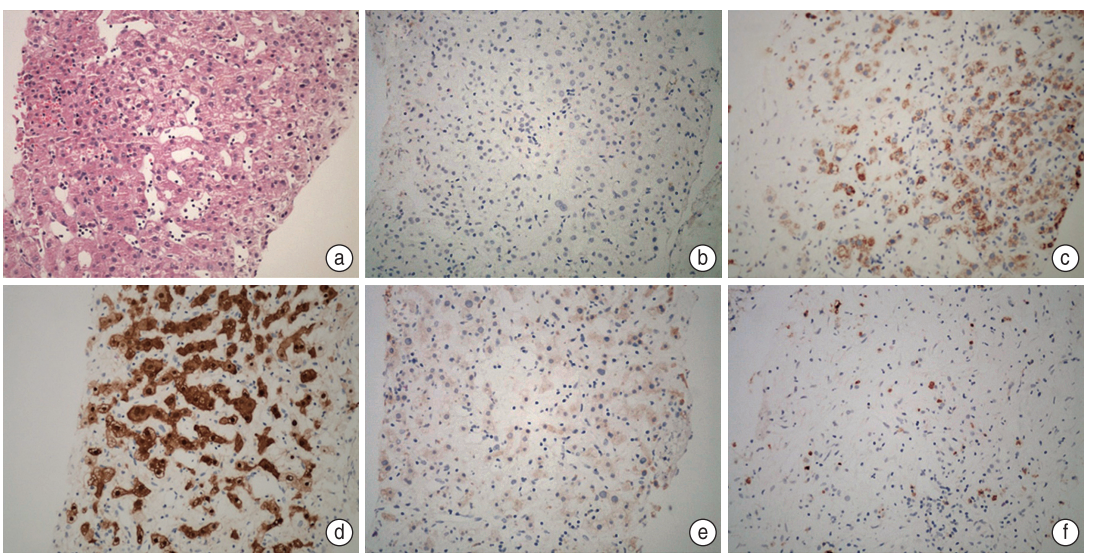

Fig. 5 HE staining of benign liver lesions and expression level of different biomarkers in benign liver lesions. (a) HE staining of benign liver lesions. HepPar-1 (c) and Arg-1 (d) were positively expressed in benign liver lesions, while GPC3 (b), CK19 (e) and EMA (f) were negatively expressed. (× 200)

liver lesions [6–9] and closely associated with tumor growth and development. High GPC3 expression levels in HCC may suggest poor differentiation, early metastasis, and poor prognosis [29]. A previous report showed that GPC3 immunostaining was positive in 78.3% (36/46) of HCCs and 72.7% (8/11) of the HCC components of CHC sections, yet negative in ICCs [30]. In our study, GPC3 immunostaining was positive in 79.2% (95/120) of HCCs and 82.4% (14/17) of CHCs, but few ICC (6/50), metastatic adenocarcinoma (2/20), and benign liver lesion (2/28) samples. Thus, GPC3 is a sensitive and specific biomarker for identifying malignant hepatic cells.

Unlike GPC3, HepPar-1 is a positive biomarker for hepatocyte differentiation that is highly expressed in both malignant and non malignant hepatic cells [31]. The rates of HepPar-1 positivity in HCC and benign liver lesions were 80.0% (96/120) and 82.1% (23/28), respectively. Due to the high level of HepPar-1 expression in CHC (70.6%, 12/17) and benign liver lesions, the specificity of HepPar-1 for HCC diagnosis was only 64.3%. Consistent with previous studies, HepPar-1 was observed in other tumor types, with 3/50 ICC cases and 3/20 metastatic

adenocarcinoma cases staining HepPar-1-postive [14, 32]. As an enzyme involved in the urea cycle, Arg-1 is a more sensitive biomarker for hepatocytes than HepPar-1 [14, 16]. The Arg-1 positivity rates in HCC and benign liver lesions were 90.0% (108/120) and 89.3% (25/28), respectively, higher than that of HepPar-1. Like HepPar-1, the specificity of Arg-1 for HCC diagnosis was only 62.6%. Our results suggest that Arg-1 was a better biomarker than HepPar-1 for distinguishing HCC from metastatic adenocarcinoma (Arg-1 was absent in all 20 metastatic liver adenocarcinoma cases) [14, 18, 23].

To identify ICC or ICC components in CHC, we performed CK19 immunostaining. CK19 is an important cytokeratin (CK) that is mainly expressed in epithelial cells, such as those in mammary gland ducts, intestinal villi, pancreatic ducts, and liver bile ducts, but not in hepatocytes [33]. It has been reported that CK19 plays a critical role in epithelial cell proliferation and differentiation. Several studies have utilized CK19 to differentiate HCC from ICC, with the CK19 positivity rate for ICC almost 90.0% [19–20, 33]. In our study, CK19 immunoreactivity was observed in 49/50 ICC cases (98.0%). EMA, another ICC-positive biomarker was also selected and analyzed. The EMA positivity rate in ICC was 96.0% (48/50 cases), with some HCC cases (27/127) also staining EMA-positive, as reported previously [19, 22, 34]. Almost all metastatic adenocarcinomas were CK19 (19/20) and EMA-positive (18/20), with the specificity of CK19 and EMA for ICC diagnosis just 62.2% and 64.9%, respectively.

In summary, GPC3 exhibited satisfactory sensitivity and specificity for HCC diagnosis. CK19 and EMA possessed adequate sensitivity for diagnosing ICC; however, their specificities were insufficient. The combination of GPC3 and CK19 or EMA may help better differentiate HCC from CHC and ICC. For the differential diagnosis of intrahepatic lesions, the single biomarkers HepPar-1 or Arg-1 could only partially suggest that the abnormality was hepatocyte-derived; thus, combining GPC3, CK19, and EMA is necessary to determine whether the disease is HCC, CHC, or a benign liver lesion. When the biomarkers GPC3, HepPar-1, Arg-1, CK19, and EMA were combined, the specificity for HCC, ICC, and CHC diagnosis increased to 98.3%, 93.0%, and 95.9%, respectively. Based on the expression features of each biomarker for liver tumor diagnosis, GPC3 was the first choice due to its high sensitivity and specificity for HCC diagnosis. The sensitivities of HepPar-1 and Arg-1 were both adequate for detecting HCC; therefore, we recommend that HepPar-1 or Arg-1 be added subjectively, with the recommended index for Arg-1 higher than that of HepPar-1 for identifying intrahepatic hepatocytes. CK19 and EMA both exhibited high sensitivity for ICC diagnosis. We recommend that CK19 and EMA be selected alternatively, with the chosen one combined with GPC3, HepPar-1, and Arg-1 to effectively differentiate HCC from ICC and CHC. There was one limitation of the GPC3/HepPar-1/Arg-1/CK19/EMA panel, since ICC and metastatic adenocarcinoma could not be differentiated well even when all biomarkers were utilized. The problem could be solved by integrating macro- and micro-pathological observations, the patients' clinical history, and other specific biomarker immunostaining.

In conclusion, we showed that the GPC3/HepPar-1/Arg-1/CK19/EMA panel of immunohistochemical biomarkers could support the diagnosis and differential diagnosis of most liver tumors, bring convenience to pathologists, and improve the accurate diagnosis and timely treatment of patients.

Conflicts of interest

The authors indicate no potential conflicts of interest.

References

1. Bosetti C, Turati F, La Vecchia C. Hepatocellular carcinoma epidemiology. Best Pract Res Clin Gastroenterol, 2014, 28: 753–770.
2. Cong WM, Bu H, Chen J, *et al*. Practice guidelines for the pathological diagnosis of primary liver cancer: 2015 update. World J Gastroenterol, 2016, 22: 9279–9287.
3. Marquardt JU, Galle PR, Teufel A. Molecular diagnosis and therapy of hepatocellular carcinoma (HCC): an emerging field for advanced technologies. J Hepatol, 2012, 56: 267–275.
4. Idikio HA. Immunohistochemistry in diagnostic surgical pathology: contributions of protein life-cycle, use of evidence-based methods and data normalization on interpretation of immunohistochemical stains. Int J Clin Exp Pathol, 2009, 3: 169–176.
5. McCourt CM, Boyle D, James J, *et al*. Immunohistochemistry in the era of personalised medicine. J Clin Pathol, 2013, 66: 58–61.
6. Hsu HC, Cheng W, Lai PL. Cloning and expression of a developmentally regulated transcript MXR7 in hepatocellular carcinoma: biological significance and temporospatial distribution. Cancer Res, 1997, 57: 5179–5184.
7. Wang F, Jing X, Wang T, *et al*. Differential diagnostic value of GPC3-CD34 combined staining in small liver nodules with diameter less than 3 cm. Am J Clin Pathol, 2012, 137: 937–945.
8. Tremosini S, Forner A, Boix L, *et al*. Prospective validation of an immunohistochemical panel (glypican 3, heat shock protein 70 and glutamine synthetase) in liver biopsies for diagnosis of very early hepatocellular carcinoma. Gut, 2012, 61: 1481–1487.
9. Zhu D, Qin Y, Wang J, *et al*. Novel glypican-3-binding peptide for *in vivo* hepatocellular carcinoma fluorescent imaging. Bioconjug Chem, 2016, 27: 831–839.
10. Capurro M, Martin T, Shi W, *et al*. Glypican-3 binds to frizzled and plays a direct role in the stimulation of canonical Wnt signaling. J Cell Sci, 2014, 127: 1565–1575.
11. Gao W, Kim H, Feng M, *et al*. Inactivation of Wnt signaling by a human antibody that recognizes the heparan sulfate chains of glypican-3 for liver cancer therapy. Hepatology, 2014, 60: 576–587.
12. Fico A, Maina F, Dono R. Fine-tuning of cell signaling by glypicans. Cell Mol Life Sci, 2011, 68: 923–929.

13. Miao HL, Pan ZJ, Lei CJ, *et al*. Knockdown of GPC3 inhibits the proliferation of Huh7 hepatocellular carcinoma cells through down-regulation of YAP. J Cell Biochem, 2013, 114: 625–631.
14. Sang W, Zhang W, Cui W, *et al*. Arginase-1 is a more sensitive marker than HepPar-1 and AFP in differential diagnosis of hepatocellular carcinoma from nonhepatocellular carcinoma. Tumour Biol, 2015, 36: 3881–3886.
15. Fujiwara M, Kwok S, Yano H, *et al*. Arginase-1 is a more sensitive marker of hepatic differentiation than HepPar-1 and glypican-3 in fine-needle aspiration biopsies. Cancer Cytopathol, 2012, 120: 230–237.
16. Nguyen T, Phillips D, Jain D, *et al*. Comparison of 5 immunohistochemical markers of hepatocellular differentiation for the diagnosis of hepatocellular carcinoma. Arch Pathol Lab Med, 2015, 139: 1028–1034.
17. Chandan VS, Shah SS, Torbenson MS, *et al*. Arginase-1 is frequently positive in hepatoid adenocarcinomas. Hum Pathol, 2016, 55: 11–16.
18. Radwan NA, Ahmed NS. The diagnostic value of arginase-1 immunostaining in differentiating hepatocellular carcinoma from metastatic carcinoma and cholangiocarcinoma as compared to HepPar-1. Diagn Pathol, 2012, 7: 149.
19. Maeno S, Kondo F, Sano K, *et al*. Morphometric and immunohistochemical study of cholangiolocellular carcinoma: comparison with non-neoplastic cholangiole, interlobular duct and septal duct. J Hepatobiliary Pancreat Sci, 2012, 19: 289–296.
20. Ryu HS, Lee K, Shin E, *et al*. Comparative analysis of immunohistochemical markers for differential diagnosis of hepatocelluar carcinoma and cholangiocarcinoma. Tumori, 2012, 98: 478–484.
21. Qin J, Wang M, Qin J, *et al*. High promoter activity of cytokeratin-19 gene in cholangiocarcinoma. Mol Clin Oncol, 2018, 9: 467–471.
22. Balitzer D, Joseph NM, Ferrell L, *et al*. Immunohistochemical and molecular features of cholangiolocellular carcinoma are similar to well-differentiated intrahepatic cholangiocarcinoma. Mod Pathol, 2019, Jun 11. doi: 10.1038/s41379-019-0290-0. [Epub ahead of print].
23. Timek DT, Shi J, Liu H, *et al*. Arginase-1, HepPar-1, and Glypican-3 are the most effective panel of markers in distinguishing hepatocellular carcinoma from metastatic tumor on fine-needle aspiration specimens. Am J Clin Pathol, 2012, 138: 203–210.
24. Wong KF, Xu Z, Chen J, *et al*. Circulating markers for prognosis of hepatocellular carcinoma. Expert Opin Med Diagn, 2013, 7: 319–329.
25. Wang Z, Yang G, Nie P, *et al*. Dynamical observation on biological progression of VX2 liver tumors to identify the optimal time for intervention in animal models. PLoS One, 2013, 8: e74327.
26. Xie S, Tang LG, Cai X, *et al*. Might liver transplantation recipients with primary hepatocellular carcinoma benefit from GVT effect of aGVHD? Chinese-German J Clin Oncol, 2014, 13: 535–538.
27. Wang L, Yao M, Yao Y, *et al*. Diagnostic and monitoring values of circulating AFP-mRNA and HS-GGT for hepatocellular carcinoma. Chinese-German J Clin Oncol, 2014, 13: 457–463.
28. Sauzay C, Petit A, Bourgeois AM, *et al*. Alpha-foetoprotein (AFP): A multi-purpose marker in hepatocellular carcinoma. Clin Chim Acta, 2016, 463: 39–44.
29. Shirakawa H, Suzuki H, Shimomura M, *et al*. Glypican-3 expression is correlated with poor prognosis in hepatocellular carcinoma. Cancer Sci, 2009, 100: 1403–1407.
30. Shirakawa H, Kuronuma T, Nishimura Y, *et al*. Glypican-3 is a useful diagnostic marker for a component of hepatocellular carcinoma in human liver cancer. Int J Oncol, 2009, 34: 649–656.
31. McKnight R, Nassar A, Cohen C, *et al*. Arginase-1: a novel immunohistochemical marker of hepatocellular differentiation in fine needle aspiration cytology. Cancer Cytopathol, 2012, 120: 223–229.
32. Fan Z, van de Rijn M, Montgomery K, *et al*. Hep par 1 antibody stain for the differential diagnosis of hepatocellular carcinoma: 676 tumors tested using tissue microarrays and conventional tissue sections. Mod Pathol, 2003, 16: 137–144.
33. Liu LZ, Yang LX, Zheng BH, *et al*. Ck7/CK19 index: A potential prognostic factor for postoperative intrahepatic cholangiocarcinoma patients. J Surg Oncol, 2018, 117: 1531–1539.
34. Kim GJ, Rhee H, Yoo JE, *et al*. Increased expression of CCN2, epithelial membrane antigen, and fibroblast activation protein in hepatocellular carcinoma with fibrous stroma showing aggressive behavior. PLoS One, 2014, 9: e105094.

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ORIGINAL ARTICLE

The prognostic potential of pretreatment C-reactive protein to albumin ratio in stage IE/IIE extranodal natural killer/T-cell lymphoma*

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Abstract

Objective The aim of this study was to determine the prognostic significance of the C-reactive protein-to-albumin ratio (CRP/Alb) for stage IE/IIE upper aerodigestive tract extranodal NK/T cell lymphoma patients.

Methods One hundred and fourteen patients diagnosed with extranodal NK/T cell lymphoma at Sichuan Cancer Hospital from September 2011 to November 2016 were retrospectively reviewed. An optimal cutoff value of CRP/Alb for overall survival rate as an endpoint was obtained using the receiver operating curve (ROC).

Results The optimal cutoff value of CRP/Alb was 0.15. For the low CRP/Alb group, the 3-year progression-free survival (PFS) was 78.6% and the 3-year overall survival (OS) was 80.7%. The 3-year PFS and OS values for the high CRP/Alb group were 41.6% and 45.2%, respectively. Differences for PFS ($P < 0.001$) and OS ($P < 0.001$) between the two groups were statistically significant. Univariate analysis showed that ECOG, IPI, CRP, GPS, and CRP/Alb were significantly associated with PFS. Similarly, all five were also significantly associated with OS. Multivariate analysis further confirmed that ECOG and CRP/Alb were independent prognostic factors for both PFS and OS. Moreover, the cutoff value of CRP/Alb showed superior prognostic ability in discriminating between patients with different outcomes in low-risk group based on GPS, IPI, and KPI scores.

Conclusion CRP/Alb is a promising prognostic marker for early-stage extranodal NK/T cell lymphoma.

Key words: C-reactive protein to albumin ratio (CRP/Alb); extranodal NK/T cell lymphoma; prognosis introduction

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Extranodal natural killer/T cell lymphoma, nasal-type (ENKTL) is a rare type of non-Hodgkin's lymphoma (NHL) characterized by highly aggressive and heterogeneous clinical features [1]. It occurs much more frequently in Asia than in Western countries (5.3% *vs* 0.3%) [2–3]. Most ENKTL tumors (about 80%) are localized to the upper aerodigestive tract, including the nasal cavity, paranasal sinus, nasopharynx, and oropharynx. It was previously known as lethal midline granuloma. Less common tumor sites (20%) are the gastrointestinal tract, skin, testis, lung, muscle, and salivary glands [4]. The vast majority of patients are diagnosed at stage IE/IIE and are sensitive to radiotherapy; however, a significant fraction of patients exhibit recurrence [5]. An International Prognostic Index (IPI) has been established for many subtypes of NHL, but its prognostic value has remained controversial for ENKTL because of the imbalanced patient distributions [6]. Given the limitations of the IPI, the Korean Prognostic Index (KPI) has been shown to have better prognostic performance than the IPI, but the KPI has not been stratified for early-stage patients [7] These prognostic models are based mainly on clinical characteristics that fail to account for our increasing understanding of the mechanisms underlying ENKTL. Therefore, a novel prognostic biomarker for these patients is urgently needed.

Recently, multiple studies have shown that inflammation plays an important role in th tumor microenvironment, with inflammation mediators (such as chemokines, cytokines, free radical) affecting tumor proliferation, progression, and metastasis [8–10]. So far, multiple investigators have reported that prognostic scores

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based on inflammatory factors, including the C-reactive protein (CRP) [11] and the Glasgow Prognostic Score (GPS) [12], have significant prognostic value for ENKTL patients. However, the prognostic value of the CRP/Alb ratio for ENKTL has not yet been examined. Therefore, we tested whether the CRP/Alb ratio could serve as a predictor of survival in early-stage ENKTL patients.

Materials and methods

Patients

One hundred and fourteen patients with newly-diagnosed upper aerodigestive tract ENKTL were identified at Sichuan Cancer Hospital from September 2011 to November 2016, using the 2016 World Health Organization criteria [13] and clinical staging according to the Ann Arbor system [14]. All study subjects met the following inclusion criteria: (1) pathologically and immunohistochemically confirmed ENKTL [1]; (2) newly diagnosed as stage I/IIE patients; (3) no antitumor therapy had been performed; (4) neither active infections nor symptoms of inflammation; and (5) follow-up data were available. Exclusion criteria for subjects in this study were as follows: (1) advanced, recurrent, or refractory ENKTL; (2) previous chemotherapy or radiotherapy; (3) obvious cardiopulmonary insufficiency.

We collected pretreatment data, including age, gender, Eastern Cooperative Oncology Group performance status (ECOG PS), B symptoms, serum lactate dehydrogenase (LDH) levels, serum C-reactive protein (CRP) levels, and serum albumin (Alb) levels. CRP was measured with a kit of Nephstar equipment (Goldsite Diagnostics Inc., China). Alb was measured with an ALB Kit (Medicalsystem Biotechnology Co. China) on a Mindray BS-820 Chemistry Analyzer (Mindray, China). To confirm tumor staging, bone marrow examination, computed tomography (CT) of the chest, abdomen, and pelvis, and/or magnetic resonance imaging (MRI) of the head and neck were performed. The IPI [6] and KPI [7] scores were calculated as previously described. The GPS score [12] was calculated from CRP and Alb measurements using standard thresholds (>10 mg/L for CRP and < 35 g/L for Alb). Patients with CRP levels above 10 mg/L or Alb levels below 35 g/L were assigned a score of 1.

Treatments

The treatment regimans were radiotherapy alone (21 patients); CHOP or CHOP-like combined with radiotherapy (26); and asparaginase combined with radiotherapy (67). Chemotherapy regimens were CHOP or CHOP-like (cyclophosphamide, doxorubicin, vincristine, prednisone or etoposide) and P-GEMOX (Peg-asparaginase, gemcitabine, oxaliplatin). Intensity-modulated radiotherapy (IMRT) was delivered using a 6-MeV linear accelerator, with a dose range of 50–62 Gy in daily fractions of 1.8-2.0 Gy, 5 d/week.

Follow-up

Overall survival (OS) was defined as the time of diagnosis until death from any cause ,or until the time of the most recent follow-up visit for surviving patients. Progression-free survival (PFS) was defined as the interval from the time of diagnosis to the time of first documented disease progression, relapse, death, or most recent follow-up visit.

Statistical analysis

The receiver operating curve (ROC) and the Youden index [maximum (sensitivity + specificity - 1)] [15] were used to determine the optimal cutoff value for CRP/Alb. Patients were divided into low and high CRP/Alb groups according to the calculated CRP/Alb cutoff value. The chi-square test was used to test for the statistical significance of correlations between CRP/Alb values and clinicopathological parameters. Survival analysis was performed using the Kaplan–Meier method and differences were evaluated using the log-rank test. Multivariate analysis was conducted by stepwise Cox regression. All data were analyzed using SPSS 17.0 software. $P < 0.05$ was considered statistically significant and all P values presented here correspond to two-sided significance tests.

Results

Patient characteristics

This study cohort included 74 males and 40 females (ratio 1.9). Median age was 45 years (range 15-84), with 19% older than 60. A majority of patients (54%) were in stage IE. LDH levels were elevated in only 28% of patients. A minority of patients (40%) presented with systemic B symptoms. The majority of patients (84%) exhibited good performance status according to ECOG scores. CRP levels over 10 mg/L were observed in 21% of patients, and 13% presented with hypoalbuminemia.

The majority of patients (75%) were classified as GPS score 0. According to IPI scores, 74% were low-risk (score 0–1). According to KPI scores, 67% were classified as low-risk (score 0–1). For the CRP/Alb score, 73% were classified as low-risk, with the remaining 27% classified as high-risk. The CRP/Alb high-risk classification was significantly correlated with elevated CRP, low Alb levels, GPS score ≥ 1, and KPI score ≥ 2 (all $P < 0.05$). The relationships between the CRP/Alb and clinical characteristics are summarized in Table 1.

Optimal cutoff value for CRP/Alb

Using overall survival rate as an endpoint, ROC analysis revealed that the optimal cutoff value of CRP/Alb ratio was 0.15 and the area under curve (AUC) was 0.712 (sensitivity = 57.1%, specificity = 84.9%) (Fig. 1a). In contrast, ROC analysis showed that GPS, IPI, and KPI were inferior predictors of OS CRP/Alb; (GPS: AUC = 0.659, sensitivity = 47.2%, specificity = 84.6%; IPI: AUC = 0.592, sensitivity = 38.9%, specificity =7 9.5%; and KPI: AUC = 0.561, sensitivity = 41.7%, specificity = 70.5%) (Fig. 1b).

Survival analysis

Follow-ups have been performed through March 2019. The overall median survival was 37.5 months (range 3–82). For all subjects, 3-year PFS was 68.1% (Fig. 2a) and 3-year OS was 70.6% (Fig. 2b). For the low-risk CRP/Alb group, the 3-year PFS was 78.6% and 3-year OS was 80.7%. For the high-risk CRP/Alb group, 3-year PFS and OS were 41.4%, and 45.2%, respectively. The result revealed that ENKTL patients in the high CRP/Alb group had significantly poorer PFS (χ^2 = 24.183, P < 0.001, Fig. 2c), and overall survival (χ^2 = 22.514, P < 0.001, Fig. 2d).

Prognostic factors for progression-free survival

Univariate analysis showed that ECOG (P = 0.002), IPI (P = 0.035), CRP (P < 0.001), GPS (P < 0.001), and CRP/Alb (P < 0.001) were significantly associated with PFS. Multivariate analysis further demonstrated that both ECOG (P = 0.033) and CRP/Alb (P = 0.018) were independent prognostic factors for PFS. The results of univariate and multivariate analysis were presented in Table 2.

Prognostic factors for overall survival

Similarly, univariate analysis revealed that ECOG (P = 0.001), LDH (P = 0.046), IPI (P = 0.022), CRP (P < 0.001), GPS (P < 0.001), and CRP/Alb (P < 0.001) were

Table 1 Relationship between the CRP/Alb and patient parameters (*n*, %)

Characteristics	Total	CRP/Alb ≤ 0.15	CRP/Alb > 0.15	χ^2	*P*
Age (years)					
≤ 60	92 (80.7)	68 (81.9)	24 (77.4)	0.295	0.587
> 60	22 (19.3)	15 (18.1)	7 (22.6)		
Gender					
Female	40 (35.1)	30 (36.1)	10 (32.3)	0.150	0.699
Male	74 (64.9)	53 (63.9)	21 (67.7)		
ECOG					
0–1	96 (84.2)	72 (86.7)	24 (77.4)	0.859	0.354
≥ 2	18 (15.8)	11 (13.3)	7 (22.6)		
B symptoms					
No	68 (59.6)	55 (66.3)	13 (41.9)	5.551	0.018
Yes	46 (40.4)	28 (33.7)	18 (58.1)		
LDH (U/L)					
≤ 240	82 (71.9)	67 (80.7)	15 (48.4)	11.688	0.001
> 240	32 (28.1)	16 (19.3)	16 (51.5)		
Clinical stage					
I	61 (53.5)	47 (56.6)	14 (45.2)	1.193	0.275
II	53 (46.5)	36 (43.4)	17 (54.8)		
CRP (mg/L)					
≤ 10	90 (78.9)	83 (100)	7 (22.6)	81.394	< 0.001
> 10	24 (21.1)	0 (0)	24 (77.4)		
ALB (g/L)					
< 35	15 (13.2)	5 (6.0)	10 (32.3)	11.395	0.001
≥ 35	99 (86.8)	78 (94.0)	21 (67.7)		
GPS score					
0	85 (74.6)	78 (94.0)	7 (22.6)	60.655	< 0.001
≥ 1	29 (25.4)	5 (6.0)	24 (77.4)		
IPI score					
0–1	84 (73.7)	65 (78.3)	19 (61.3)	3.373	0.066
≥ 2	30 (26.3)	18 (21.7)	12 (38.7)		
KPI score					
0–1	76 (66.7)	62 (74.7)	14 (45.2)	8.861	0.003
≥ 2	38 (33.3)	21 (25.3)	17 (54.8)		

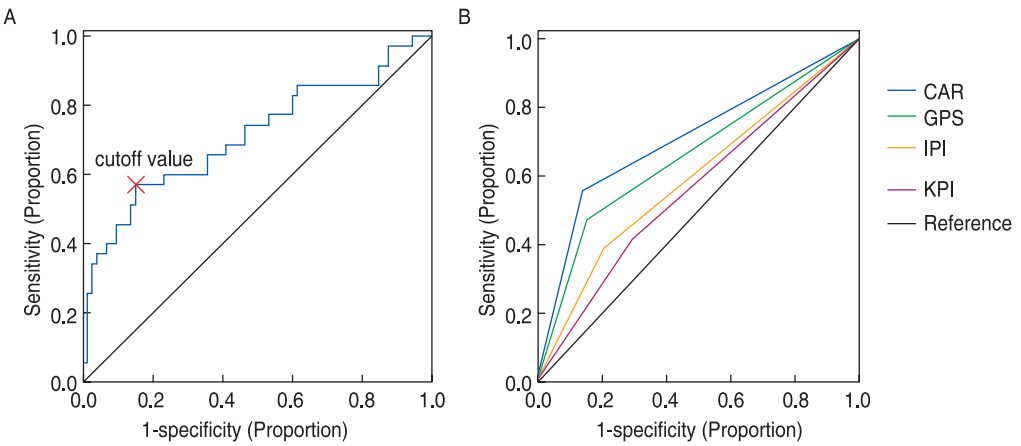

Fig. 1 The receiver operating curves analysis: (a) the optimal cut-off value for CRP/Alb determined by ROC; (b) The comparison among CRP/Alb, Glasgow Prognostic Score (GPS), International Prognostic Index (IPI), and Korean Prognostic Index (KPI) by ROC

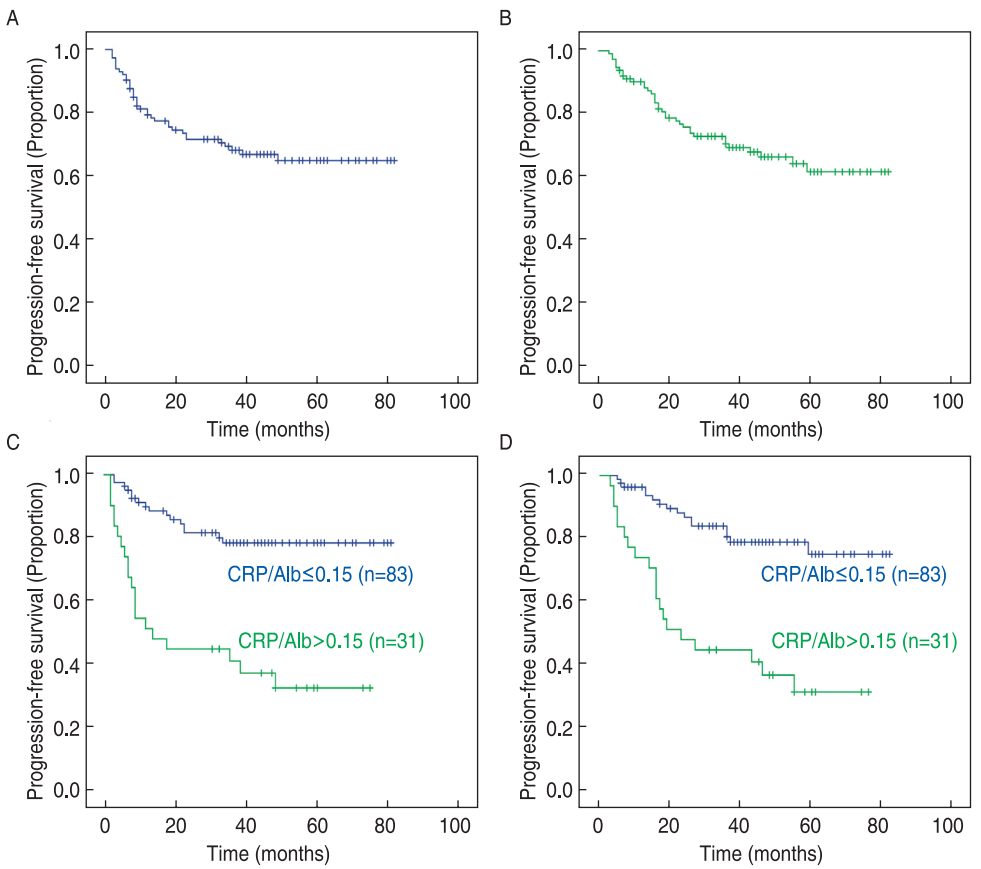

Fig. 2 Kaplan-Meier curves depicting the difference in survival: (a) The whole patients for PFS; (b) The whole patients for OS; (c) PFS of patients CRP/Alb ≤ 0.15 vs CRP/Alb > 0.15; (d) OS of patients CRP/Alb ≤ 0.15 vs CRP/Alb > 0.15

significantly related to OS. Multivariate analysis further revealed that ECOG ($P = 0.027$) and CRP/Alb ($P = 0.025$) were independent prognostic factors for OS. These results were shown in Table 3.

Prognostic value of combining CRP/Alb with GPS, IPI, and KPI

Based on GPS scores, 75% of cases were placed in the low-risk group (score 0), which could not be further

Table 2 Prognostic factors for progression-free survival (PFS)

Clinical characteristics		Univariate analysis (PFS)		Multivariate analysis (PFS)		
		χ^2	*P*	HR	95% CI	*P*
Age (years)	≤ 60 *vs* > 60	0.215	0.643			
Gender	Male *vs* Female	0.728	0.393			
ECOG score	0-1 *vs* ≥ 2	9.714	0.002	4.150	1.124–15.318	0.033
B symptoms	No *vs* Yes	0.483	0.487			
Clinical stage	IE *vs* IIE	2.596	0.107			
LDH (U/L)	≤ 240 *vs* > 240	3.275	0.070			
IPI score	0–1 *vs* ≥ 2	4.451	0.035	0.596	0.171–2.079	0.417
KPI score	0–1 *vs* ≥ 2	1.388	0.239			
CRP (mg/L)	≤10 *vs* > 10	19.210	< 0.001	1.010	0.101–10.132	0.993
GPS score	0 *vs* ≥ 1	13.713	< 0.001	1.244	0.161–9.623	0.834
CRP/Alb	≤ 0.15 *vs* > 0.15	24.183	< 0.001	3.818	1.262–11.549	0.018

Table 3 Prognostic factors for overall survival

Clinical characteristics		Univariate analysis (PFS)		Multivariate analysis (PFS)		
		χ^2	*P*	HR	95% CI	*P*
Age (years)	≤ 60 *vs* > 60	0.241	0.624			
Gender	Male *vs* Female	0.668	0.414			
ECOG score	0–1 *vs* ≥ 2	10.478	0.001	4.482	1.184–16.970	0.027
B symptoms	No *vs* Yes	0.391	0.532			
Clinical stage	IE *vs* IIE	2.772	0.096			
LDH (U/L)	≤ 240 *vs* > 240	3.995	0.046	1.159	0.490–2.741	0.736
IPI score	0–1 *vs* ≥ 2	5.270	0.022	0.597	0.159–2.247	0.446
KPI score	0–1 *vs* ≥ 2	1.614	0.204			
CRP (mg/L)	≤ 10 *vs* > 10	18.048	< 0.001	0.983	0.095–10.198	0.988
GPS score	0 *vs* ≥ 1	13.265	< 0.001	1.234	0.147–10.356	0.847
CRP/Alb	≤ 0.15 *vs* > 0.15	22.514	< 0.001	3.623	1.176–11.165	0.025

distinguished differences low-risk group. According to the IPI score, 74% of patients were classified as low-risk (score 0–1), which also failed to discriminate between patients with different outcomes. Similarly, by KPI score 67% were placed in the group (score 0–1), which also was unsatisfactory. However, when CRP/Alb was added to all three of the aforementioned three models, we obtained significantly better discrimination: GPS score 0 (PFS: χ^2 = 7.932, P = 0.005, Fig. 3a; OS: χ^2 = 7.326, P =0.007, Fig. 3b); IPI score 0–1 (PFS: χ^2 = 19.743, P < 0.001, Fig. 3c; OS: χ^2 = 17.210, P < 0.001, Fig. 3d); and KPI score 0-1 (PFS: χ^2 = 24.971, P < 0.001, Fig. 3e; OS: χ^2 = 20.495, P < 0.001, Fig. 3f).

Discussion

ENKTL is a distinct subtype of NHL characterized by prominent vascular destruction, tissue necrosis, and inflammatory cell infiltration [1]. Despite recent improvements resulting from combining asparaginase with radiotherapy for early-stage ENKTL patients, there remain patients with poor prognoses [16–17]. Therefore, a novel, powerful predictor for those patients is needed. A diverse set of studies have been devoted to elucidating the link between inflammation and cancer, evidence to support inflammatory cells, proinflammatory cytokines, and chemokines in the tumor microenvironment promoted tumor cell growth, proliferation, development and metastasis, resistance to treatment, leading to worse survival and prognosis [8–10]. A previous study introduced the CRP/Alb ratio as an inflammation-based prognostic ratio for patients with acute medical admissions and sepsis [18]. A recent meta-analysis demonstrated that pretreatment CRP/Alb was correlated with poor survival in multiple types of solid tumors [19]. Some studies have shown that C-reactive protein and GPS scores are independent prognostic factors for ENKTL patients [11–12]. However, the mechanisms by which the CRP/Alb ratio might be related to survival have remained unclear. Several potential explanations might account for this.

CRP, an important acute-phase response protein, is produced mainly by hepatocytes. Elevated CRP level

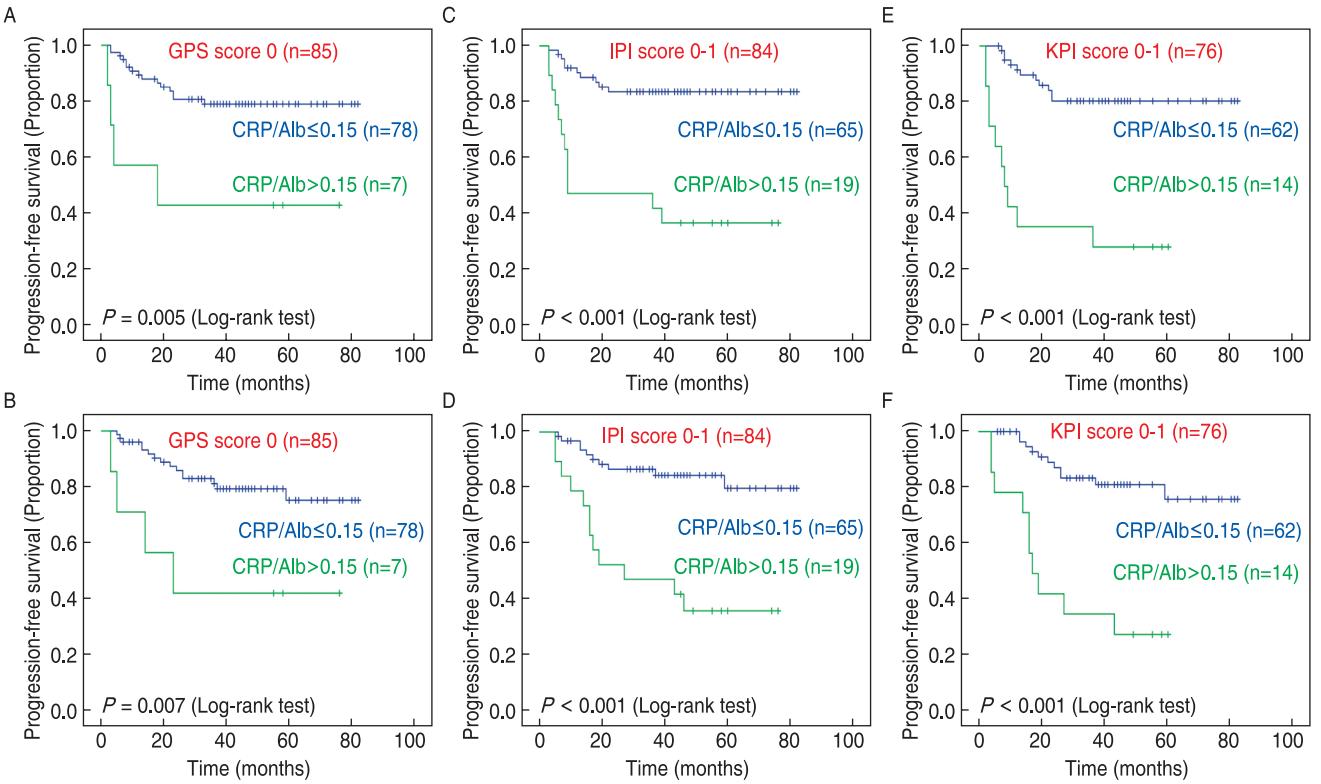

Fig. 3 Subgroup survival analysis for prognostic value of combing CRP/Alb with GPS, IPI, and KPI: (A) PFS of GPS score 0 patients for CRP/Alb ≤ 0.15 vs CRP/Alb > 0.15. (B) OS of GPS score 0 patients for CRP/Alb ≤ 0.15 versus CRP/Alb > 0.15. (C) PFS of IPI score 0–1 patients for CRP/Alb ≤ 0.15 vs CRP/Alb > 0.15. (D) OS of IPI score 0–1 patients for CRP/Alb ≤ 0.15 vs CRP/Alb > 0.15. (E) PFS of KPI score 0–1 patients for CRP/Alb ≤ 0.15 vs CRP/Alb > 0.15. (F) OS of KPI score 0–1 patients for CRP/Alb ≤ 0.15 vs CRP/Alb > 0.15

is a marker for an inflammatory response secondary to infection, cell injury, tissue destruction, and/or tumor necrosis. It is regulated by proinflammatory cytokines, especially interleukin-6, secreted by innate immune cells. In addition, increases in CRP levels result in activation of inflammatory pathways that might trigger DNA mutation and inhibit apoptosis, leading to tumor development and progression. Previous studies have demonstrated that persistent inflammation often exists in the tumor microenvironment, inducing increases in the levels of multiple cytokines, including interleukin-6, tumor growth factors, and CRP. These cytokines in turn promote tumor cell proliferation, invasiveness, metastasis, suppression of the antitumor immune response, all of which have large effects on the response to treatment [22–23]. Studies have shown that serum elevated CRP was positively associated with the solid cancer [24–26], including ENKTL [11]. Therefore, the underlying mechanisms of the relationship between elevated CRP and poor prognosis need to be better understood.

Albumin is also produced by the liver, which helps to maintain intravascular oncotic pressure, facilitate the transport of substances, and scavenge free radicals. Hypoalbuminemia is not merely a consequence of inflammation, but also can reflect a patient's nutritional state [27]. There are several possible mechanisms that could underlie the association between decreased albumin and cancer. First, hypoalbuminemia may reflect a systemic inflammatory response triggered by production of proinflammatory cytokines from the tumor itself, restraining the ability of the liver to generate albumin and promoting acute-phase protein synthesis [28]. Second, the release of cytokines from inflammatory cells increases microvascular permeability, increasing the flow of serum albumin toward the extravascular compartment [29]. Third, serum albumin may provide an indication that mirrors some other unfavorable status of the host, such as functional impairment or immunosuppression. There is evidence that supplement of some trophic factors improves immune function in cancer patients [30]. The presence of a systemic inflammatory response and a concomitant nutritional decline may make hard for patients to bear treatment toxicity. If that is the case, serum albumin level may be an appropriate index for evaluation and prediction of nutritional improvements for cancer patients in clinical practice. Recently, accumulating evidence have shown that hypoalbuminemia was correlated with poor survival in many cancer patients [31–34].

By the chi-square test, we found that the CRP/Alb ratio was strongly associated with important clinical factors including B symptoms, elevated LDH, and the KPI score (Table 1), suggesting that high CRP/Alb is correlated with more aggressive tumor behaviors or high tumor burden. There also were positive correlations between high CRP/Alb ratios and elevated CRP, low serum albumin levels, and the GPS score. Univariate analysis showed that LDH, ECOG, and IPI scores were associated with PFS or OS in ENKTL patients (Table 2, Table 3), consistent with previous studies [6–7, 11–12].

In accordance with previous studies [11–12], CRP and GPS scores were significantly associated with poor survival in ENKTL patients in univariate analyses. However, in the our study, CRP and GPS scores were not independent prognostic factors in multivariate analysis. Two possible explanations for this difference might be that CRP and GPS both use the same two biomarkers, or that using the ratio of CRP/Alb better captures the interplay between the two markers than the simple category assignment method. By contrast to a previous report [12], no significant correlation was observed between clinical stage and B symptoms by univariate analysis. The reason for this might be that all patients had localized lesions that were sensitive to radiotherapy, which is very effective in treating in early stage ENKTL patients. Li *et al* [11–12] demonstrated that age was an independent prognostic factor for survival. By contrast, we observed no significant correlation between age and survival. The explanation might be that our older patients could tolerate treatment better, because of progress in the optimization of chemotherapy and radiotherapy technology.

Finally, we analyzed whether our new prognostic model was equivalent or superior to other validated prognostic models. The GPS score [12] is one of the best-demonstrated inflammation-based prognostic scores for ENKTL, so it was our primary benchmark. Most surprising was that when classified by the GPS, 75% of our patients were in the score 0 group (Table 1), meaning that GPS had no prognostic power for the majority of our patients. Similar to GPS score, the CRP/Alb ratio is calculated with the same values (CRP and albumin). Thus, when we categorized patients into two groups and explored their survival differences, we found that CRP/Alb identified a group of patients with a GPS score of 0 (Fig. 3a–3b). Therefore, the CRP/Alb ratio could be used in combination with the GPS score to better predict survival. The IPI score is an important prognostic score in patients with NHL, but its predictive value was not perfect in ENKTL [6] because of the imbalanced distribution of patients in risk groups. Consistent with previous studies [6–7], we found that 74% of our patients were in the IPI score 0–1 group (low to low-intermediate risk) and 67% were in the KPI score 0–1 group. In this way, both IPI and KPI failed to further discriminate among patients within these groups. However, when the CRP/Alb ratio was added to IPI or KPI, the low- to low-intermediate risk group patients were separated into two groups with significantly different survival outcomes (Fig. 3c–3f). In summary, the CRP/Alb ratio had better prognostic ability in discriminating among lower-risk patients than did the GPS, IPI, or KPI scores.

Conclusions

The CRP/Alb ratio is a simple, feasible and inexpensive prognostic biomarker for ENKTL. This ratio showed superior prognostic ability in discriminating between patients with different outcomes in the low-risk group than the more established prognostic scores, GPS, IPI and KPI. The limitations of our study were its small sample size, its retrospective nature, and heterogeneity in treatment regimens. Therefore, larger, multicenter prospective studies are needed to confirm the prognostic value of the CRP/Alb ratio and to provide a better understanding of the mechanisms underlying it.

Conflicts of interest

The authors declare that they have no competing interests.

References

1. Tse E, Kwong YL. The diagnosis and management of NK/T-cell lymphomas. J Hematol Oncol, 2017, 10: 85–85.
2. Liao Y, Du XB, Zou QF, Extranodal natural killer/T-cell lymphoma,nasal type: epidemiology study. Oncol Transl Med (Chinese), 2012, 11: 290–293.
3. Haverkos BM, Pan Z, Gru AA, *et al*. Extranodal NK/T cell lymphoma, nasal type (ENKTL-NT): an update on epidemiology, clinical presentation, and natural history in north american and european cases. Curr Hematol Malig Rep, 2016, 11:514–527.
4. Tse E, Kwong YL. How I treat NK/T-cell lymphomas. Blood, 2013, 121: 4997–5005.
5. Hua W, Pengfei L, Xinke Z, *et al*. Histological vascular invasion is a novel prognostic indicator in extranodal natural killer/T-cell lymphoma, nasal type. Oncol Lett, 2016, 12: 825-836.
6. Chim CS, Ma SY, Au WY, *et al*. Primary nasal natural killer cell lymphoma: long-term treatment outcome and relationship with the international prognostic index. Blood, 2004, 103: 216–221.
7. Au WY, Weisenburger DD, Intragumtornchai T, *et al*. Clinical differences between nasal and extranasal natural killer/T-cell lymphoma: a study of 136 cases from the International Peripheral T-Cell Lymphoma Project. Blood, 2009, 113: 3931–3937.
8. Grivennikov SI, Greten FR, Karin M. Immunity, Inflammation, and Cancer. Cell, 2010, 140: 883–899.
9. Schreiber RD, Old LJ, Smyth MJ. Cancer immunoediting: integrating immunity's roles in cancer suppression and promotion. Science, 2011, 331: 1565–1570.
10. Fernandes JV, Cobucci RN, Jatobá CA, *et al*. The Role of the Mediators of Inflammation in Cancer Development. Pathol Oncol Res, 2015, 21: 527–534.

11. Li YJ, Li ZM, Xia Y, *et al*. Serum C-reactive protein (CRP) as a simple and Independent prognostic factor in extranodal natural killer/T-cell lymphoma, nasal type. PLoS ONE, 2013, 8: e64158.
12. Li Y, Jiang WQ, Huang JJ, *et al*. The Glasgow Prognostic Score (GPS) as a novel and significant predictor of extranodal natural killer/T-cell lymphoma, nasal type. Am J Hematol, 2013, 88: 394–399.
13. Swerdlow SH, Campo E, Pileri SA, *et al*. The 2016 revision of the World Health Organization classification of lymphoid neoplasms. Blood, 2016,127: 2375–2390.
14. Cheson BD, Fisher RI, Barrington SF, *et al*. Recommendations for initial evaluation, staging, and response assessment of Hodgkin and non-Hodgkin lymphoma: the Lugano classification. J Clin Oncol, 2014, 32: 3059–3068.
15. Kumar R, Indrayan A. Receiver operating characteristic (ROC) curve for medical researchers. Indian Pediatr, 2011, 48: 277–287.
16. Wei W, Wu P, Li L, *et al*. Effectiveness of pegaspargase, gemcitabine, and oxaliplatin (P-GEMOX) chemotherapy combined with radiotherapy in newly diagnosed, stage IE to IIE, nasal-type, extranodal natural killer/T-cell lymphoma. Hematology, 2017, 22: 320–329.
17. Li JW, Li YJ, Zhong MZ, *et al*. Efficacy and tolerance of GELOXD/P-GEMOXD in newly diagnosed nasal-type extranodal NK/T-Cell lymphoma: A multicenter retrospective study. Eur J Haematol, 2018,100: 247–256.
18. Ranzani OT, Zampieri FG, Forte DN, *et al*. C-Reactive Protein/Albumin Ratio Predicts 90-Day Mortality of Septic Patients. PLoS ONE, 2013, 8: e59321.
19. Wu J, Tan W, Chen L, *et al*. Clinicopathologic and prognostic significance of C-reactive protein/albumin ratio in patients with solid tumors: an updated systemic review and meta-analysis. Oncotarget, 2018, 9: 13934–13947.
20. Wang CS, Sun CF. C-reactive protein and malignancy: clinico-pathological association and therapeutic implication. Chang Gung Med J, 2009, 32: 471–482.
21. Heikkil K, Harris R, Lowe G, *et al*. Associations of circulating C-reactive protein and interleukin-6 with cancer risk: findings from two prospective cohorts and a meta-analysis. Cancer Causes Control, 2009, 20:15–26.
22. Allin KH, Nordestgaard BG. Elevated C-reactive protein in the diagnosis, prognosis, and cause of cancer. Crit Rev Clin Lab Sci, 2011, 48: 155–170.
23. Lee S, Choe JW, Kim HK, *et al*. High-sensitivity C-reactive protein and cancer. J Epidemiol, 2011, 21:161–168.
24. Dossus L, Jimenezcorona A, Romieu I, *et al*. C-reactive protein and postmenopausal breast cancer risk: results from the E3N cohort study. Cancer Causes Control, 2014, 25: 533–539.
25. Shrotriya S, Walsh D, Bennani-Baiti N, *et al*. C-reactive protein is an important biomarker for prognosis tumor recurrence and treatment response in adult solid tumors: a systematic review. Plos One, 2015, 10: e0143080.
26. Li J, Jiao X, Yuan Z. C-reactive protein and risk of ovarian cancer A systematic review and meta-analysis. Medicine, 2017, 96: e7822.
27. Esper DH, Harb WA. The cancer cachexia syndrome: a review of metabolic and clinical manifestations. Nutr Clin Pract, 2005, 20: 369–376.
28. McMillan, Donald C. Systemic inflammation, nutritional status and survival in patients with cancer. Curr Opin Clin Nutr Metab Care, 2009, 12: 223–226.
29. Fanali G, Masi AD, Trezza V, *et al*. Human serum albumin: From bench to bedside. Mol Aspects Med, 2012, 33: 209–290.
30. Long H, Yang H, Lin Y, *et al*. Fish oil-supplemented parenteral nutrition in patients following esophageal cancer ssurgery: effect on inflammation and immune function. Nutr Cancer, 2013, 65: 71–75.
31. Asher V, Lee J, Bali A. Preoperative serum albumin is an independent prognostic predictor of survival in ovarian cancer. Med Oncol, 2012, 29: 2005–2009.
32. Haskins IN, Baginsky M, Amdur RL, *et al*. Preoperative hypoalbuminemia is associated with worse outcomes in colon cancer patients. Clin Nutr, 2016, 36: 1333–1338.
33. Hardt J, Pilz L, Magdeburg J, *et al*. Preoperative hypoalbuminemia is an independent risk factor for increased high-grade morbidity after elective rectal cancer resection. Int J Colorectal Dis, 2017, 32: 1439–1446.
34. Ouyang X, Dang Y, Zhang F, *et al*. Low Serum Albumin Correlates with Poor Survival in Gastric Cancer Patients. Clin lab, 2018, 64: 239–245.

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ORIGINAL ARTICLE

Expression and clinical significance of CD90 and CD177 tumor stem cell markers in cervical cancer

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Abstract

Objective To investigate the expression and clinical significance of CD90 and CD177 in cervical cancer.
Methods Cases of cervical cancer (n = 102), cervical intraepithelial neoplasia (CIN, n = 52), and benign uterine disease (n = 50) were selected. The positive rates of CD90 and CD177 in the cervical tissues were detected, and the significance of CD90 and CD177 expression was analyzed.
Results The positive rate of CD90 in normal cervical tissue, CIN, and cervical cancer was 3.7%, 36.5%, and 79.4% respectively. The respective positive rates of CD177 were 1.8%, 32.7%, and 74.5%. The positive rates of CD90 and CD177 in cervical cancer tissues were the highest, followed by CIN tissues (P < 0.05). Multivariate analysis showed that pathological grade, lymph node metastasis, and tumor diameter were independent risk factors affecting the expression of CD90 and CD177 (each P < 0.05). There was a moderate positive correlation between CD90 and CD177 expression (r = 0.679, P = 0.003). The overall survival rate of 102 patients with cervical cancer was 64.7%. There were 33 deaths in the CD90 positive group and 3 in the negative group. The overall survival rates were 59.3% and 85.7% in the CD90 and negative group, respectively. There were 33 deaths in the CD177 positive group and 3 in the negative group. The overall survival rates were 56.6% and 88.5%, respectively. The difference was statistically significant.
Conclusion The expression of CD90 and CD177 has some adverse effects on the clinicopathological parameters of cervical cancer. The positive expression of CD90 and CD177 is a risk factor for poor prognosis.

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Key words: CD90; CD177; cervical cancer

Cervical cancer is one of the most common malignant tumors in women. With the gradual changes in female sexual attitudes and living habits, the incidence of morbidity has become significantly greater in younger women [1]. Tumor stem cells are a group of special tumor cells that can maintain the vitality of tumor cell populations through self-renewal and infinite proliferation, and are currently a research hotspot of tumor-targeted therapy [2–3]. CD90 and CD177 are commonly used markers of cancer stem cells, but no reports exist on the differential expression of CD90 and CD177 in normal cervical tissues, cervical intraepithelial neoplasia (CIN) tissues, and cervical cancer tissues.

Presently, 102 cases of cervical cancer, 52 cases of CIN, and 50 cases of benign uterine disease were studied to investigate the expression of the two in different cervical lesions and the clinical pathological parameters and prognosis of cervical cancer, in order to compare the relationship between cancer stem cells and cervical cancer. The study provides more experimental evidence.

Materials and methods

Research subjects

Patients with cervical cancer and CIN admitted to the Third People's Hospital in Yancheng from January 2015 to December 2017 were enrolled. Inclusion criteria included diagnoses by pathological means, initial diagnosis, surgical treatment, stage I or stage IIa, and the lack of anti-tumor therapy, such as radiotherapy and chemotherapy, before surgery. Exclusion criteria included history of malignant

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tumors and incomplete follow-up information. Finally, 52 patients with CIN and 102 patients with cervical cancer were included in the study. As well, 54 patients with benign diseases, such as uterine fibroids, were treated as normal controls.

Reagents and immunohistochemical staining

Rabbit anti-human CD90, rabbit anti-human CD177 polyclonal antibody, and SP kit were purchased from TaKaRa Bio (Shiga, Japan). Tissue was preserved in the pathology department of our hospital, and involved formaldehyde fixation, paraffin embedding, and routine dewaxing and hydration after sectioning. High temperature repair antigen, phosphate buffered saline (PBS) rinse, blocking antibody. Fifty microliters of rabbit anti-human CD90 polyclonal antibody was added dropwise and incubated overnight. The secondary antibody was added dropwise after PBS washing, and the reaction occurred in the presence of 3,3'-diaminobenzidine (DAB) was developed. Brown particles were used as a positive standard.

Follow-up

The patients were followed-up by clinic visits and telephone contact. The follow-up period began with radical surgery for cervical cancer. The deadline was January 31, 2019. Overall survival (OS) was defined as the time from radical surgery to death from any cause.

Statistical analyses

All data analyses were performed using SPSS 20.0 software (SPSS Inc., Chicago, IL, USA). The measurement data is expressed as frequency and rate, with statistical inference based on the chi-square test. Survival analysis was performed using Kaplan-Meier analysis and the log-rank test was used to compare the effects of CD90 and CD177 expression on OS. The test level of significance was $\alpha = 0.05$.

Results

Expression of CD90 and CD177 in cervical tissues

Both CD90 and CD177 were mainly expressed in the cell membrane of tumor cells, with a small amount of expression in the cytoplasm. The positive rate of CD90

Table 1 Positive rates of CD90 and CD177 in different cervical tissues

Index	Normal cervical (n = 54)	CIN (n = 52)	Cervical cancer (n = 102)	χ^2	P
CD90				85.314	0.000
–	52	33	21		
+	2	19	81		
CD177				79.628	0.000
–	53	35	26		
+	1	17	76		

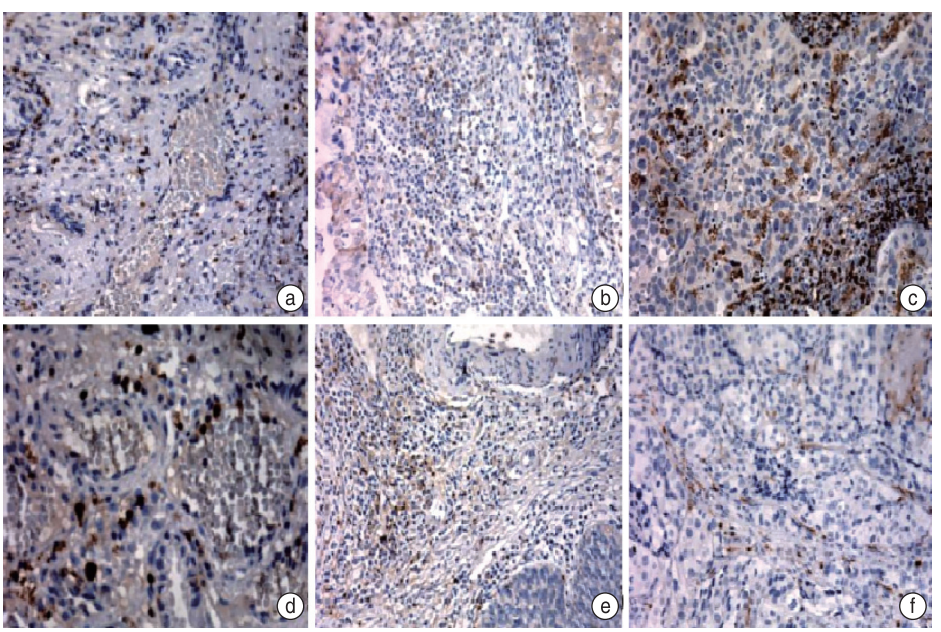

Fig. 1 Expression of CD90 and CD177 in different cervical tissues. (a) CD90 is expressed in normal cervical tissues; (b) CD90 is expressed in CIN tissues; (c) CD90 is expressed in cervical cancer tissues; (d) CD177 is expressed in normal cervical tissues; (e) CD177 is expressed in CIN tissues; (f) CD177 is expressed in cervical cancer tissues

in normal cervical, CIN, and cervical cancer tissues was 3.7% (2/54), 36.5% (19/52), and 79.4% (81/102), respectively. It was 1.8% (1/54), 32.7% (17/52), and 74.5% (76/102), respectively. After pairwise comparison, CD90 and CD177 displayed the highest positive rates in cervical cancer tissues, followed by CIN tissue, with the lowest positive rate in normal tissues. The difference was statistically significant ($P < 0.05$) (Table 1, Fig. 1).

Univariate analysis of the influence of CD90 and CD177 expression on pathological parameters of cervical cancer

Univariate analysis showed that CD90 and CD177 expression had significant effects on pathological grade, lymph node metastasis, and tumor diameter (each $P < 0.05$), but had no significant correlation with age, histological type, FIGO stage (each $P > 0.05$) (Table 2).

Multivariate analysis of the influence of expression of CD90 and CD177 on cervical cancer

All the significant indicators in the univariate analysis were included in the logistic risk model. Pathological grade, lymph node metastasis, and tumor diameter were independent risk factors for the expression of CD90 and CD177 (each $P<0.05$) (Table 3 and Table 4).

Correlation analysis of CD90 and CD177 expression in cervical cancer tissues

Spearman rank correlation test showed a positive correlation between CD90 and CD177 expression (r=0.679, P=0.003) (Table 5).

Table 2 Univariate analysis of the effects of CD90 and CD177 expression on pathological parameters of cervical cancer

index	CD90		χ^2	P	CD177		χ^2	P
	–	+			–	+		
Age (years)			0.031	0.861			0.106	0.745
≤ 45	9	33			10	32		
> 45	12	48			16	44		
Histological Type			0.038	0.845			0.039	0.843
Adenocarcinoma	4	17			5	16		
Squamous cell carcinoma	17	64			21	60		
FIGO stage			1.757	0.185			3.740	0.053
I	13	37			17	33		
II	8	44			9	43		
Pathological classification			22.985	0.000			7.607	0.022
Well-differentiated	9	4			7	6		
Moderately differentiated	9	41			13	37		
Poorly differentiated	3	36			6	33		
Lymphatic metastasis			9.520	0.002			9.177	0.002
Yes	0	27			1	26		
No	21	54			25	50		
Tumor diameter (cm)			7.708	0.005			4.444	0.035
< 3	14	27			15	26		
≥ 3	7	54			11	50		

Table 3 Effect of CD90 expression on pathological parameters of cervical cancer

Index	β	SE	Wald	95%CI	OR	P
Pathological classification	1.372	0.417	10.823	1.741–8.929	3.943	< 0.001
lymphatic metastasis	1.322	0.408	10.455	1.686–8.345	3.767	< 0.001
Tumor diameter	0.897	0.451	12.298	1.013–5.936	2.492	< 0.001

Table 4 Effect of CD177 expression on pathological parameters of cervical cancer

Index	β	SE	Wald	95%CI	OR	P
Pathological classification	1.398	0.208	18.342	2.692–6.084	4.211	< 0.001
Lymphatic metastasis	1.213	0.429	17.216	1.415–7.798	2.869	< 0.001
Tumor diameter	1.581	0.378	17.356	2.317–10.195	4.829	< 0.001

Table 5 Correlation analysis of CD90 and CD177 expression in cervical cancer tissues

CD90	CD177		*r*	*P*
	–	+		
–	6	15		
+	20	61	0.679	0.003
Total	26	76		

Effects of CD90, CD177 expression on the prognosis of patients with cervical cancer

The follow-up deadline was January 2019. Of the 102 patients with cervical cancer, 36 died and 66 survived, representing a survival rate of 64.7%. There were 33 deaths in the CD90 positive group and 3 deaths in the negative group. The OS rate for the CD90 positive and negative group was 59.3% and 85.7%, respectively. The difference was statistically significant (χ^2 = 4.606, P = 0.031). There were 33 deaths in the CD177 positive group and 3 deaths in the negative group. The respective OS rates were 56.6% and 88.5%. The difference was statistically significant (χ^2 = 5.166, P = 0.023). The results are showed in Fig. 2.

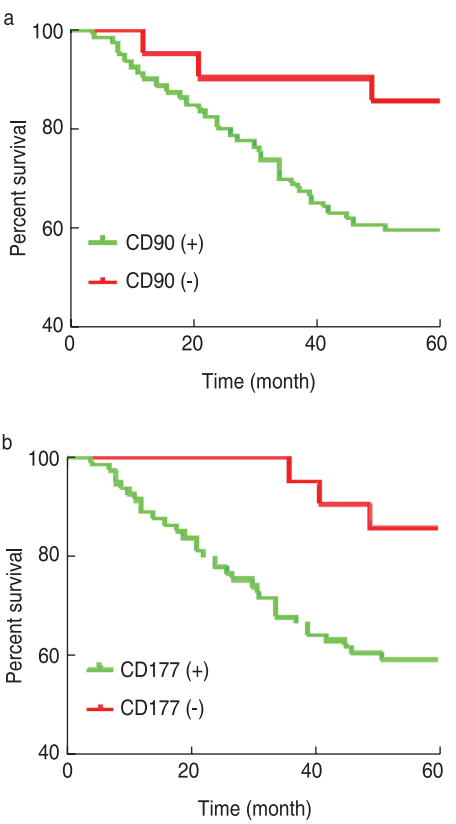

Fig. 2 Survival curves of patients with different CD90 and CD177 expression. (a) CD90 (+), CD90 (–) patient survival curve; (b) CD177 (+), CD177 (–) patient survival curve

Discussion

Cervical cancer refers to the malignant tumor that occurs at the junction with the squamous epithelial cells of the cervix, or transition zone and the columnar epithelial cells of the endocervix of the cervix. The death rate from cervical cancer is the fourth highest overall in China, and is the second leading cause of death in females [4]. Surgery is the preferred method of treatment for cervical cancer, especially in patients with early stage I or IIa [5]. Most (80%) of early cancer patients can achieve good survival through surgical treatment, but a small number of patients will relapse or metastasize in a short period of time even after standard radical surgery [6]. At the same time, for most patients with malignant tumors, radiotherapy and chemotherapy and biological immunotherapy can kill most tumor cells, but these approaches cannot fundamentally cure the tumor. This may be related to the presence of tumor cells with stem cell properties in the circulating blood [7].

Tumor stem cells have the characteristics of self-renewal, heterogeneity, and infinite proliferation, which can efficiently produce tumor cells. Surface molecules, such as CD44 and CD199, are commonly used molecules for screening cancer stem cells [8]. In cervical cancer, current research focuses on CD133, Bmi-1, p63, Oet3/4, and other molecules [9]. CD44 and CD199 are widely distributed; they are multi-molecular forms of membrane integrin, including extracellular, transmembrane, and cytoplasmic regions, which are mainly involved in mediating the interaction between cells and cells (between extracellular matrices) [10]. The main functions of the two include [11] mediating cell-to-cell adhesion by interacting with fibronectin and collagen, auxiliary or direct involvement in the uptake and degradation of hyaluronic acid, participation in lymphocyte homing, and promotion of T cell activation.

These aspects have been extensively studied in studies of gastric cancer, colon cancer, and prostate cancer, but little research has been done in cervical cancer. In this study, we found that CD90 and CD177 had the highest positive rate in cervical cancer tissues, followed by CIN tissue, with the lowest positive rate in normal tissues. The difference was statistically significant. This is consistent with the expression of malignant markers in progressive lesions. Multivariate logistic analysis showed that pathological grade, lymph node metastasis, and tumor diameter were independent risk factors for the expression of CD90 and CD177. As the pathological grade worsened, tumor malignancy increased and cell proliferation accelerated. As the expression of CD90 and CD177 increased, the degree of differentiation of cervical cancer was progressively reduced, consistent with previous findings [12]. The diameter of the tumor can directly reflect

the tumor burden of the whole body of the patient. The higher the expression of CD90 and CD177, the higher the tumor burden of the patient. Based on the study of the influence of the expression on tumor diameter and differentiation, we followed the patients and found that the OS rate of the 102 patients with cervical cancer was 64.7%. The OS rate of the CD90-positive group was 59.3%, and that of the CD177-positive group was 56.6%. The difference was statistically significant compared with the corresponding negative group, indicating that the positive expression of both has a significant adverse effect on the survival of patients.

In summary, the expression of CD90 and CD177 has a certain adverse effect on the clinicopathological parameters of cervical cancer, and the positive expression of both is a risk factor for poor prognosis. However, this study is a single-center study that used a less sensitive immunohistochemistry method. Multi-center combination, increased sample size, and improved detection technology are needed to provide conclusive clinical evidence.

Conflicts of interest

The authors declare that they have no competing interests.

References

1. Zou Y, Liu FY, Wu J, *et al*. Mutational analysis of the RAS/RAF/MEK/ERK signaling pathway in 260 Han Chinese patients with cervical carcinoma. Oncol Lett, 2017, 14: 2427–2431.
2. Sun B, Zhang D, Zhao N, *et al*. Epithelial-to-endothelial transition and cancer stem cells: two cornerstones of vasculogenic mimicry in malignant tumors. Oncotarget, 2017, 8: 30502–30510.
3. Chen D, Wu M, Li Y, *et al*. Targeting BMI1+ cancer stem cells overcomes chemoresistance and inhibits metastases in squamous cell carcinoma. Cell Stem Cell, 2017, 20: 621–634.
4. Veluchamy JP, Heeren AM, Spanholtz J, *et al*. High-efficiency lysis of cervical cancer by allogeneic NK cells derived from umbilical cord progenitors is independent of HLA status. Cancer Immunol Immunother, 2017, 66: 51–61.
5. Zhang J, Liu J, Zhu C, *et al*. Prognostic role of vascular endothelial growth factor in cervical cancer: a meta-analysis. Oncotarget, 2017, 8: 24797–24803.
6. Guo M, Zhao X, Yuan X, *et al*. MiR-let-7a inhibits cell proliferation, migration, and invasion by down-regulating PKM2 in cervical cancer. Oncotarget, 2017, 8: 28226–28236.
7. Lee YJ, Kim DY, Lee SW, *et al*. A postoperative scoring system for distant recurrence in node-positive cervical cancer patients after radical hysterectomy and pelvic lymph node dissection with para-aortic lymph node sampling or dissection. Gynecol Oncol, 2017, 144: 536–540.
8. Sahin N, Akatli AN, Celik MR, *et al*. The role of CD90 in the differential diagnosis of pleural malignant mesothelioma, pulmonary carcinoma and comparison with calretının. Pathol Oncol Res, 2017, 23: 487–491.
9. Jin X, Chen X, Hu Y, *et al*. LncRNA-TCONS_00026907 is involved in the progression and prognosis of cervical cancer through inhibiting miR-143-5p. Cancer Med, 2017, 6: 1409–1423.
10. Zhang K, Che S, Su Z, *et al*. CD90 promotes cell migration, viability and sphere-forming ability of hepatocellular carcinoma cells. Int J Mol Med, 2018, 41: 946–954.
11. Lu Z, Chen H, Zheng XM, *et al*. Expression and clinical significance of high risk human papillomavirus and invasive gene in cervical carcinoma. Asian Pac J Trop Med, 2017, 10: 195–200.
12. Ohno T, Noda SE, Okonogi N, *et al*. In-room computed tomography-based brachytherapy for uterine cervical cancer: results of a 5-year retrospective study. J Radiat Res, 2017, 58: 543–551.

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ORIGINAL ARTICLE

Expression and significance of PAX8 gene in ovarian cancer based on Oncomine database Meta-analysis

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Abstract

Objective Although great progress has been made in the diagnosis and treatment of ovarian cancer, this disease is still the leading cause of death due to female reproductive system tumors. It has been reported that the paired box 8 (*PAX8*) gene is involved in the occurrence and development of a variety of human tumors. However, few researchers have investigated this phenomenon in detail.

Methods Here, the BioGPS database was used to analyze the expression of the *PAX8* gene in normal tissues. The Oncomine database was used to search for *PAX8* gene information, and the findings were analyzed via a meta-analysis with regard to the significance of this gene in ovarian cancer. The Kaplan-Meier Plotter database was used to analyze the prognosis of patients with ovarian cancer. The Cancer Cell Line Encyclopedia (CCLE) was used only for obtaining cell line analysis data regarding the *PAX8* gene.

Results The relevant results of the BioGPS database analysis showed that *PAX8* is not expressed or under-expressed in normal ovarian tissues. Oncomine data showed 454 different results; there were 417 study samples in total, with 9 results showing a significant statistical difference in *PAX8* expression, 5 of which were related to high expression of *PAX8* and 4 of which were related to low *PAX8* expression. Cell line analysis data of the PAX8 gene obtained from CCLE showed high expression in ovarian cancer, which is consistent with the high expression of *PAX8* in ovarian cancer research found using the Oncomine database. The Kaplan-Meier Plotter database showed that the expression level of PAX8 had a significant effect on the overall survival time of patients ($P = 0.042$). Compared with the low expression group, the overall survival time of ovarian cancer patients in the high expression group of PAX8 was significantly low ($P < 0.05$).

Conclusion Through an in-depth study of the gene information of ovarian cancer-related genes using the gene chip data in the Oncomine database, it was concluded that PAX8 is highly expressed in ovarian cancer tissues and directly correlates to the prognostic survival of ovarian cancer patients. These findings provide an important basis for the development of clinical gene-targeted cancer therapeutic drugs.

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Key words: ovarian cancer; gene; paired box 8; cancer cell line encyclopedia

Ovarian cancer (OC) presents a serious threat to women's health and is considered the third greatest threat to female reproductive health following cervical and endometrial cancer. Due to the difficulty that exists in its early detection and the lack of effective of prognosis determination, it has the highest mortality among the most common reproductive cancers [1–3], not only posing a great economic burden to patients' families and society, but also putting significant pressure on the patients' physical and mental state.

With the development of a social economy and the progress of medical science, technology, instrumentation, and diagnostic methods, the incidence of OC has improved significantly [4]. In addition, technology for tumor detection is in development. By studying the mechanism of OC development at the molecular level and determining the genes highly expressed in OC, further research and development of new drugs targeting these genes can be conducted, thus raising the survival prognosis of patients with OC [5]. The paired box 8 (*PAX8*) gene plays an important role in promoting the development of the Müllerian system in female reproductive organs. It

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is a restricted cell transcription factor, which also exerts additional effects on OC tissue [6–7]. The *PAX8* gene was first described by scholars in the study of Müllerian tube source tumors with differentiating gastrointestinal metastatic carcinoma [8–9] and showed that *PAX8* is highly expressed OC tissue and not expressed in digestive system tumors [10–12]. The *PAX8* gene is highly sensitive and specific in the diagnosis of tumors in the reproductive system [13–14]. However, there is no systematic study that has been conducted on the *PAX8* gene in OC.

This study uses the Oncomine database, BioGPS database, Kaplan-Meier Plotter database, and Cancer Cell Line Encyclopedia (CCLE) to conduct a meta-analysis of PAX8 expression in OC tissue to assist further research on the role of PAX8 in the occurrence, development, and prognosis of OC.

Materials and methods

BioGPS database analysis

The expression of PAX8 gene in normal human tissues was analyzed by using the BioGPS database platform (http://biogps.org).

Oncomine database analysis

The Oncomine database (http://www.oncomine.org) currently collects 715 gene expression data sets and 86,733 cancer tissue and normal tissue samples, and the number of genes is increasing. The database is comprised of multiple gene chip databases and integrated databases, and is currently the largest database platform in the world. Through this database, gene differential expression analysis, co-expression analysis, and meta-analysis of various common cancer tissues and normal tissues can be achieved. The Oncomine database platform meets the need for gene chips by setting constraints. The settings for this research study were: (1) "Cancer Type: Ovarian Cancer"; (2) "Gene: *PAX8*"; (3) "Data Type: mRNA"; (4) "Analysis Type: Cancer *vs.* Normal Analysis"; (5) Threshold setting conditions (P value < 1E-4, fold change > 2, gene rank = top 10%). The corresponding results are described in a box chart.

Cancer cell line encyclopedia database analysis

The Cancer Cell Line Encyclopedia (https://portals.broadinstitute.org/) was used for cell analysis of *PAX8* genes.

Kaplan-meier plotter database analysis

An online survival analysis was performed by using the Kaplan-Meier Plotter database (http://kmplot.com/analysis/). The screening conditions were as follows: (1) "Cancer: Ovarian Cancer"; (2) "Gene: *PAX8*"; (3) "Survival: PFS"; (4) "All."

Results

PAX8 gene expression in normal human tissues

Results from the BioGPS database analysis showed that *PAX8* was not expressed or under-expressed in normal ovarian tissue. Although its high expression was found in thyroid tissue, low expression or non-expression was found in the other tissues of the body (Fig. 1).

Expression of PAX8 in common tumor types

Oncomine showed data for 454 items of different types. Of these, there were 9 tumor types presenting statistically significant *PAX8* expression levels; 5 of which demonstrated high expression levels of *PAX8*, while 4 demonstrated low expression levels (Fig. 2).

Expression of PAX8 in OC

Based on the Oncomine database results, it can be determined that, since 2004, a total of 5 studies involving the comparison of PAX8 expression in OC tissues and normal tissues were conducted. In total, 417 samples—including ovarian serous carcinoma and ovarian clear cell carcinoma—were compared with those of normal tissue. Such articles are published in journals, such as the British Journal of Cancer [15], Cancer Research [16–17], Cancer Science [18], and Clinical Cancer Research [19]. For the meta-analysis using the Oncomine database, the 5 relevant research results showed that, compared with the normal group, the PAX8 gene had a median rank of 27.0 among all the differentially expressed genes (P = 1.350E-5), meaning that the PAX8 gene was highly expressed in OC (Fig. 3).

Differential expression of PAX8 in different OC gene chips

By using gene chip analysis, various studies [15-19] have shown that PAX8 expression in ovarian tumors is higher than that in the normal tissues (P < 0.001), especially in ovarian serous adenocarcinoma, and it is significantly higher in ovarian clear cell carcinoma (Fig. 4).

Results from the CCLE analysis of PAX8 genes

Results from the CCLE analysis of *PAX8* genes show that in cancerous tissue, 37 classes of *PAX8* gene-expressing cell lines exist, showing different degrees of expression; OC ranked third place, confirming an increased expression in ovarian tumor tissue. These findings comply with the high expression of PAX8 in OC seen in the Oncomine database (Fig. 5).

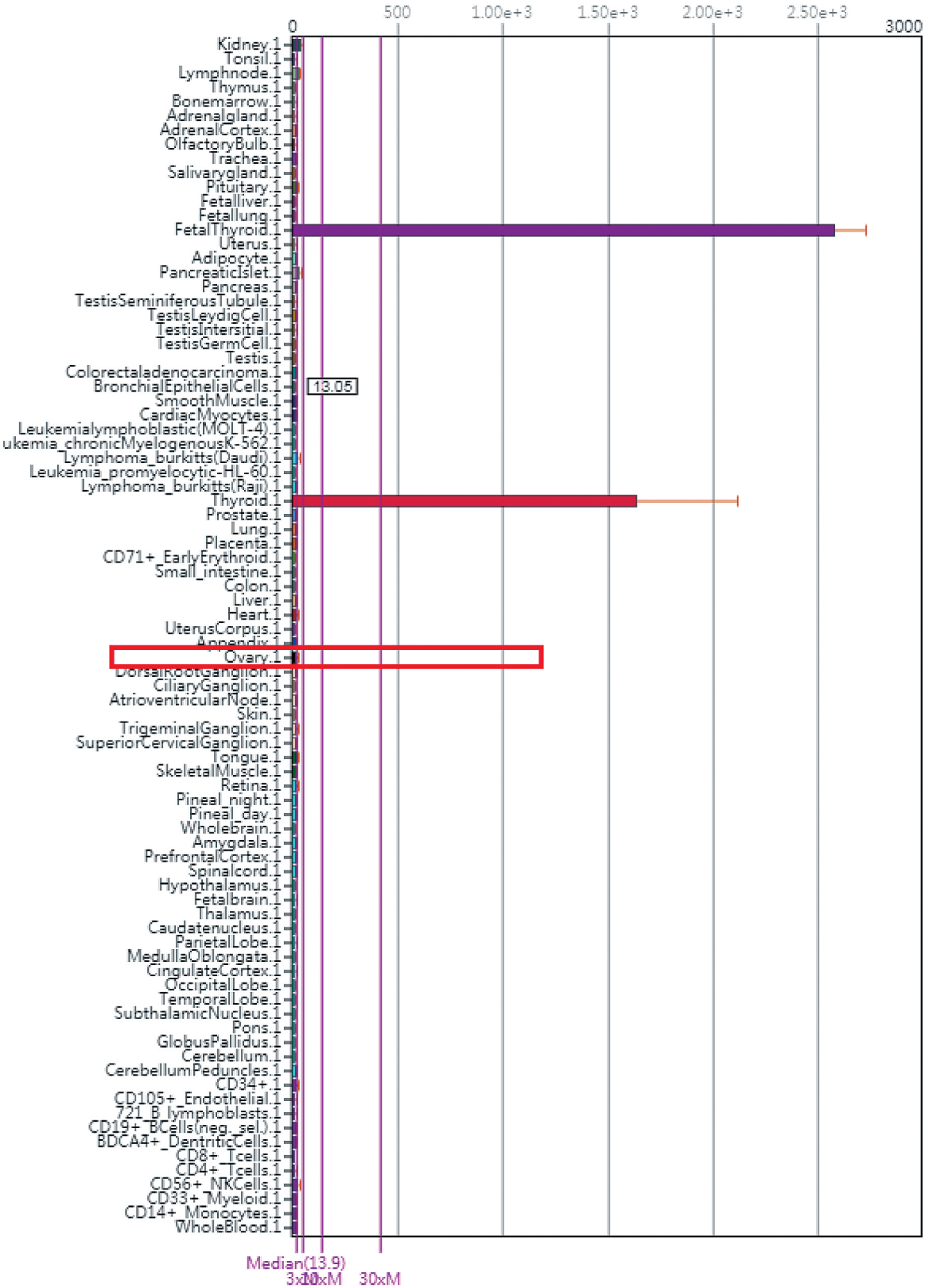

Fig. 1 Expression of PAX8 in normal human tissues

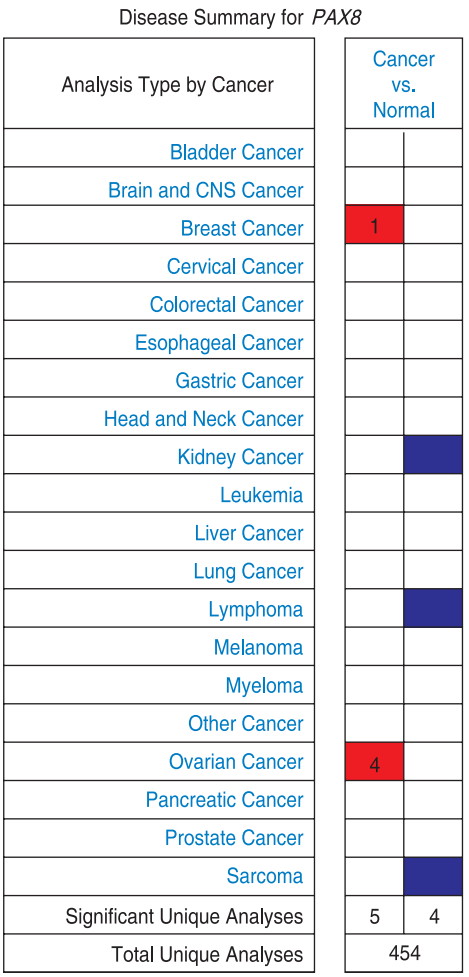

Fig. 2 Expression of the PAX8 gene in all tumor types

Relationship between PAX8 and survival prognosis of OC patients

Results from the Kaplan-Meier Plotter database showed that the expression level of *PAX8* had a significant effect on the total survival time of patients (P = 0.042). Compared with the low expression group, the total survival time of patients in the group with high PAX8 expression was significantly reduced (Fig. 6).

Discussion

OC, as one of the most common malignancies in the world, has a high incidence in female reproductive systems and a corresponding high mortality rate. According to epidemiological statistics, the incidence of OC is increasing slightly [20–21]. Meanwhile, the survival prognosis of OC is very poor; the 5-year survival rate is less than 30%. However, as these tumors have been studied at the molecular level, gene-targeting therapy has become one of the most promising treatments for tumors at present. With the increase in research concerning OC at the molecular level, it has been found that CP, WFDC2, CELSR2, and several other genes associated with OC are of great importance for improving the prognosis of the patients suffering from OC, and are also key factors in the future development of gene-targeting drugs [22–23]. Therefore, the study of key genes related to the occurrence, development, and prognosis of OC has clinical significance for the treatment of OC and the improvement of the survival prognosis of patients suffering from it, which has been a hot topic in recent years.

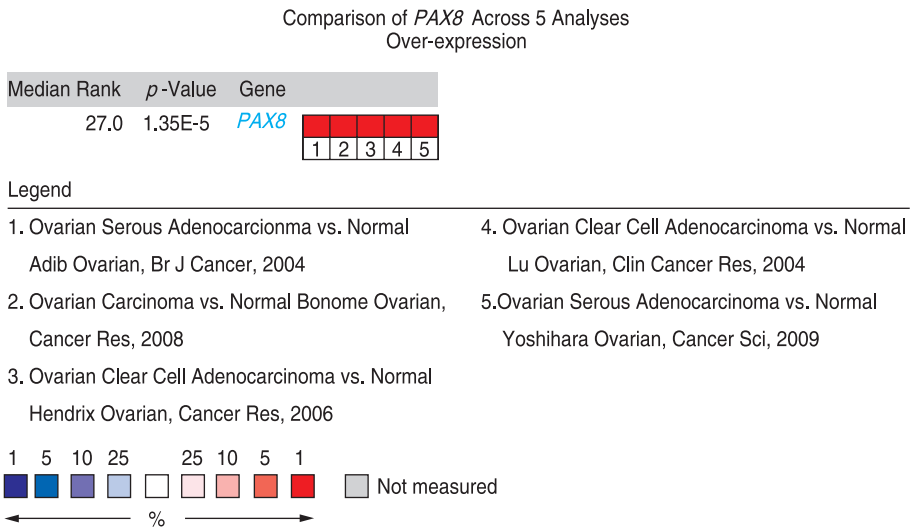

Fig. 3 Summary expression of PAX8 in ovarian cancer research

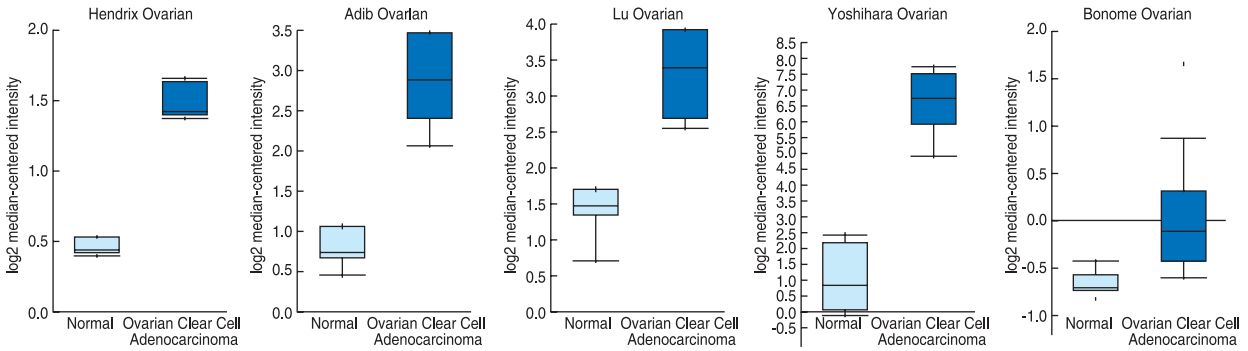

Fig. 4 Expression of PAX8 in different ovarian cancer research chips

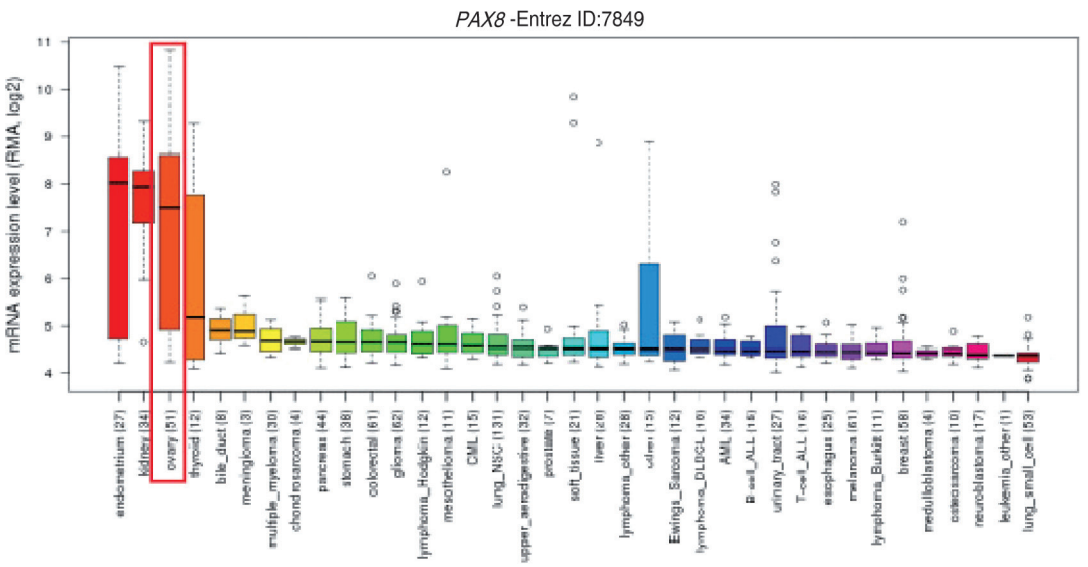

Fig. 5 Results of CCLE analysis of PAX8 gene in cell lines

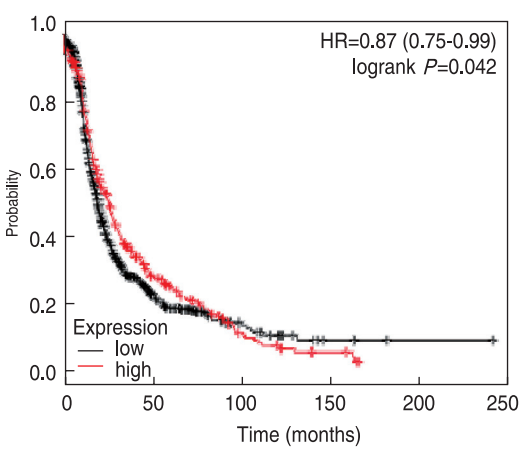

Fig. 6 Relationship between PAX8 expression and prognosis of ovarian cancer survival

PAX8 is a member of the paired box (PAX) family of transcription factors. The family has 9 members; all PAX proteins are composed of 128 amino acids and are located on chromosome 2q13. *PAX8* can attach to specific regions of DNA, having an impact on transcription and gene regulation and control. *PAX8* can control the development of ovarian tissue epithelium and epithelial tumors in the female genital tract with high expression. Moreover, the positive expression rate is low in mucous ovarian tumors, while it is negative in any other benign or malignant tumors of stomatal cells [12]. At present, the exact function of this gene is not quite clear. However, it has been found that *PAX8* has a significant correlation with the development and prognosis of ovarian cancer [24]. Therefore, *PAX8* is a highly specific tumor marker gene that can be used for detecting OC. This is also evident given the high expression of *PAX8* in OC based on the data retrieved from the Oncomine database.

Although previous studies have shown *PAX8* expression increased in various tumor cells and OC groups, the environment and the independent study sample size in these studies was not sufficient, which could easily have led to sampling error. Therefore, the credibility of these researches is not high. The Oncomine database is the world's largest database of gene chip data, which includes information from various tumor gene chips; moreover, all users worldwide have free access to it. The expression of *PAX8* in various tumor tissues can be found by utilizing the Oncomine database. Specifically, the *PAX8* gene was highly expressed in ovarian cancer tissue. In total, there were 9 studies with statistically significant results; among them, *PAX8* showed high expression in 5 studies and low expression in 4 studies. For Oncomine *PAX8* genes, the database was used for meta-analysis. Further results showed high *PAX8* expression in 417 cases among the study samples, with particularly high expression in OC. Moreover, CCLE *PAX8* gene expression data were applied for analysis in OC tissues and cells and showed increased expression. The prognosis of OC patients was analyzed using the Kaplan-Meier Plotter database. The Kaplan-Meier Plotter database is the most extensive database platform covering tumor genes in the world, including 1,816 OC samples. It can be used to analyze prognosis using 54,675 gene chips, and the related retrieval results are reliable. Based on the Kaplan-Meier Plotter database retrieval concerning the *PAX8* gene, it was found that its gene expression and overall prognosis for OC was significantly related to lifecycle ($P = 0.042$), and inclusive outcomes showed that the survival time of patients with high expression of PAX8 noticeably decreased ($P < 0.05$). This consequence may be due to the fact that the PAX8 protein is associated with abnormal regulation of epithelial cells in the ovary, resulting in the occurrence of tumors.

There are numerous sources of gene chip data in this study. Its sample size is large, and the relevant method is highly consistent. The differences in sample size may lead to certain errors; however, this has no impact on the authenticity and credibility of the overall result.

In summary, through the in-depth exploration of the expression data of *PAX8* in OC using the Oncomine database, it has been found that *PAX8* is highly expressed in OC and is directly related to the prognosis of patients with OC. The large sample size of this study helps to avoid the inherent drawback of single-method research and sample size errors and may be of great importance to the treatment of clinical OC.

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We are grateful to the contributors of data: Oncomine, Kaplan-Meier Plotter, BioGPS, and Cancer Cell Line Encyclopedia.

Conflicts of interest

The authors declare no potential conflicts of interest.

References

1. Kirchhoff C, Habben I, Ivell R, *et al*. A major human epididymis-specific cDNA encodes a protein with sequence homology to extracellular proteinase inhibitors. Biol Reprod, 1991, 45: 350–357.
2. Brightwell R, Eng K, Lele S. Use of hematologic biomarkers during chemotherapy predicts survival in ovarian cancer patients. Eur J Gynaecol Oncol, 2017, 38: 378–381.
3. Wang K, Gan L, Jeffrey E, *et al*. Monitoring gene expression profile changes in ovarian carcionomas using cDNA microarray.Gene,1999, 229: 101–108.
4. Zhu YF, Gao GL, Tang SB, *et al*. Effect of WFDC2 silencing on the proliferation, motility and invasion of human serous ovarian cancer cells in vitro. Asian Pac J Trop Med, 2013, 6: 265–272.
5. Granato T, Porpora MG, Longo F, *et al*. HE4 in the differential diagnosis ofovarian masses. Clin Chim Acta, 2015, 446: 147–155.
6. Cheung HW, Cowley GS, Weir BA, *et al*. Systematic investigation of genetic vulnerabilities across cancer cell lines reveals lineage -specific dependencies in ovarian cancer. Proc Natl Acad Sci U S A, 2011, 108: 12372–12377.
7. Mhawech-Fauceglia P, Wang D, Samrao D, *et al*. Pair Box 8 (PAX8) protein expression in high grade, late stage (stages III and IV) ovarian serous carcinoma. Gynecol Oncol, 2012, 127: 198–201.
8. Levanon K, Ng V, Piao HY, *et al*. Primary *ex vivo* cultures of human fallopian tube epithelium as a model for serous ovarian carcinogenesis. Oncogene, 2010, 29: 1103–1113.
9. Tong GX, Devaraj K, Hamele-Bena D, *et al*. PAX8: a marker for carcinoma of Mullerian origin in serous effusions. Diagn Cytopathol, 2010, 39: 567–574.
10. Ordonez NG. Value of Pax 8 immunostaining in tumor diagnosis: a review and update. Adv Anat Pathol, 2012, 19: 140–151.
11. Nonaka D, Chiriboga L, Soslow RA. Expression of pax8 as a useful marker in distinguishing ovarian carcinomas from mammary carcinomas. Am J Surg Pathol, 2008, 32: 1566–1571.
12. Ordonez NG. Value of PAX8, PAX2, claudin-4, and hcaldesmon immunostaining in distinguishing peritoneal epithelioid mesotheliomas from serous carcinomas. Mod Pathol, 2013, 26: 553–562.
13. Ozcan A, Shen SS, Hamilton C, *et al*. Pax 8 expression in non-neoplastic tissues, primary tumors, and metastatic tumors: a comprehensive immunohistochemical study. Mod Pathol, 2011, 24: 751–764.
14. Laury AR, Perets R, Piao H, *et al*. A comprehensive analysis of Pax8 expression in human epithelial tumors. Am J Surg Pathol, 2011, 35: 816–826.
15. Adib TR, Henderson S, Perrett C, *et al*. Predicting biomarkers for ovarian cancer using gene-expression microarrays. Br J Cancer, 2004, 90: 686–692.
16. Hendrix ND, Wu R, Kuick R, *et al*. Fibroblast growth factor 9 has oncogenic activity and is a downstream target of Wnt signaling in ovarian endometrioid adenocarcinomas. Cancer Res, 2006, 66: 1354–1362.
17. Bonome T, Levine DA, Shih J, *et al*. A gene signature predicting for survival in suboptimally debulked patients with ovarian cancer. Cancer Res, 2008, 68: 5478–5486.
18. Yoshihara K, Tajima A, Komata D, *et al*. Gene expression profiling of advanced stage serous ovarian cancers distinguishes novel

subclasses and implicates ZEB2 in tumor progression and prognosis. Cancer Sci, 2009, 100: 1421–1428.
19. Lu KH, Patterson AP, Wang L, *et al*. Selection of potential markers for epithelial ovarian cancer with gene expression arrays and recursive descent partition analysis. Clin Cancer Res, 2004,10: 3291–3300.
20. Gasiorowska E, Walkowiak GP, Warchoł W, *et al*. Ovarian cancer and normal fallopian tube high WFDC2 expression does not correlate with HE4 serum level. Ginekol Pol, 2015, 86: 335–339.
21. Choe SR, Kim YN, Park CG, *et al*. RCP induces FAK phosphorylation and ovarian cancer cell invasion with inhibition by curcumin. Exp Mol Med, 2018, 50: 52.
22. Bauersfeld SP, Kessler CS, Wischnewsky M, *et al*. The effects of shortterm fasting on quality of life and tolerance to chemotherapy in patients with breastand ovarian cancer: a randomized cross-over pilot study. BMC Cancer, 2018, 18: 476.
23. Hentze JL, Høgdall C, Kjær SK, *et al*. Searching for new biomarkers in ovarian cancer patients: Rationale and design of a retrospective study under the Mermaid III project. Contemp Clin Trials Commun, 2017, 8: 167–174.
24. Peters DG, Kudla DM, Deloia JA, *et al*. Comprarative gene expression analysis of ovarian carcinoma and normal ovarian epithelium by serial analysis of gene expression. Cancer Epidemiol Biomarkers Prev, 2005, 14: 1717–1723.

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ORIGINAL ARTICLE

Analysis of cancer incidence and mortality data in Heilongjiang province cancer registries, China, 2015

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Abstract

Objective In recent years, the rising incidence of cancer has increased patients' living and economic burdens. This study analyzed the incidence and death due to malignant tumors in tumor registries in Heilongjiang province (China) in 2015 to provide a scientific basis for the prevention and treatment of malignant tumors in this province.

Methods Data on tumor incidence and patient deaths were collected from seven tumor registries in Heilongjiang province (China) in 2015. According to the stratification of urban and rural areas and patient sex, the crude, standard, and accumulative rates (0–74 years of age) were calculated. The 2000 China Population Census data and Segi's standard population were used to calculate the age-standardized rates.

Results In 2015, the incidence rate of malignant tumors in Heilongjiang cancer registries was 259.90/100 000. The age-standardized incidence rates in the Chinese and world standard populations were 158.89/100 000 and 155.06/100 000, respectively, with a cumulative incidence rate (0–74 years) of 17.68%. The incidence of malignant tumors in urban areas was 273.55/100 000, while that in rural areas was 220.32/100 000. The incidence of malignant tumors in men was 270.89/100 000, higher than that in women (249.04/100 000). Lung cancer had the highest incidence, followed by breast cancer, liver cancer, colorectal cancer, and thyroid cancer. The mortality rate of malignant tumors in Heilongjiang cancer registries was 164.69/100 000. The age-standardized mortality rates in Chinese and in world standard populations were 95.29/100 000 and 94.35/100 000, respectively, with a cumulative mortality rate (0–74 years) of 10.44%. The mortality rate of malignant tumors in urban areas was 169.51/100 000, while that in rural areas was 150.72/100 000. The mortality rate of malignant tumors in men was 201.64/100 000, higher than that in women (128.21/100 000). Lung cancer had the highest mortality, followed by liver cancer, stomach cancer, colorectal cancer, and breast cancer.

Conclusion Lung, liver, breast, and colorectal cancers were the most common cancers in Heilongjiang province, China, and should be considered the key cancer types for prevention and treatment. Moreover, the incidence of thyroid cancer is increasing, and thus early preventative measures should be implemented.

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Key words: tumor registration; incidence; mortality; Heilongjiang province, China

Rapid economic development in Heilongjiang province (China) in recent years has resulted in significantly improved standards of living. In this province, smoking, excessive drinking, and lack of exercise are the main risk factors for chronic diseases [1]. In addition to the aggravation of aging, malignant tumors have become the main disease affecting the health of residents in Heilongjiang province, China. Tumor registration is an internationally recognized standard method to objectively collect information on the incidence and death due to tumors in the population, provide data for formulating health plan guidelines and policies, and offer basic theoretical data [2]. The Heilongjiang Cancer Center, Harbin, China is responsible for surveillance of the incidence and mortality of malignant tumors in the province via annual collection, auditing, evaluation, analysis, and timely publication of registration data. The present study assessed data on the incidence and death of malignant tumors in Heilongjiang province, China, in 2015. Following auditing and evaluation, qualified

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registry data were selected for analysis and reported as follows.

Materials and methods

Data sources

Nine registries in Heilongjiang province (China) reported data on cancer incidence, death, and the corresponding population database. The study period was January 1, 2015, to December 31, 2015, and included confirmed new cases of malignant tumors (ICD10: C00.0–C97, D45–D47) and benign tumors of the central nervous system (ICD10: D32.0–D33.9). Deaths due to malignant tumors recorded in the cancer registries during the study period were also obtained.

Quality evaluation

According to the Guidelines for Cancer Registration in China (2016) [3], the Cancer Incidence in Five Continents Volume IX, and the requirements for registration of the International Cancer Research Center/International Association for Cancer Registration [4–5], statistical software was used to examine and evaluate the data. The integrity, validity, and timeliness of the data were evaluated by main indicators such as percentage of morphologically verified cases (MV%), percentage of death certificates only (DCO%), and mortality-to-incidence ratio (M/I).

The quality evaluation criteria of the cancer registration data received by the National Cancer Center in China were 55% < MV% < 95%, 0.55% < M/I < 0.85, and DCO% < 20%. The data reported in the present study were assessed and audited according to these requirements. A total of seven cancer registration areas were included, including four urban areas (Nangang District, Harbin City; Xiangfang District, Harbin City; Daoli District, Harbin City; and Mudanjiang City) and three rural areas (Shangzhi City, Boli County, and Hailin City).

The seven cancer registration areas included 4 985 087 registered residents (2 476 644 men and 2 508 443 women), of which 3 706 516 were in urban areas and 1 278 571 in rural areas, accounting for 13.12% of the registered population in Heilongjiang province (China) in 2015. The total MV%, DCO%, and M/I of cancer registration areas in Heilongjiang province (China) were 78.98%, 2.01%, and 0.63, respectively. These indicators suggested that the data had good integrity and reliability.

Statistical analysis

Crude incidence and mortality, sex- and age-specific incidences and mortalities, age-standardized rates (ASR), and cumulative and truncated rates were analyzed. The top 10 malignant tumors in morbidity and mortality were described. Segi's world population and the China 2000 Population census data were used as the population standards.

Results

Incidence of malignant tumors

In 2015, the crude incidence of malignant tumors was 259.90/100 000 (270.89/100 000 men and 249.04/100 000 women), while the age-standardized incidence rates in Chinese standard (ASIRC) and world standard (ASIRW) populations were 158.89/100 000 and 155.06/100 000 respectively, with a cumulative rate (0–74 years of age) of 17.68%.

The crude incidence of malignant tumors in urban cancer registration areas was 273.55/100 000 (283.89/100 000 men and 263.54/100 000 women), while the ASIRC and ASIRW were 160.32/100 000 and 156.41/100 000 respectively, corresponding to a cumulative rate (0–74 years) of 17.95%. The crude incidence of malignant tumors in rural cancer registration areas was 220.32/100 000 (234.67/100 000 men and 205.30/100 000 women). The ASIRC and ASIRW were 156.42/100 000 and 152.72/100 000, respectively, and the cumulative rate (0-74 years) was 16.97%. Compared to that in rural areas, the total incidence among men and women in urban areas was higher than that in rural areas (Table 1).

Table 1 Incidence of malignant tumors in Heilongjiang province, China, 2015

Area	Gender	New cases	Crued incidence (1/10^5)	ASIRC (1/10^5)	ASIRW (1/10^5)	Cumulative rate (0–74) (%)
Total	Both	12956	259.90	158.89	155.06	17.68
	Male	6709	270.89	169.35	168.46	19.89
	Female	6247	249.04	151.30	144.57	15.85
Urban	Both	10139	273.55	160.32	156.41	17.95
	Male	5174	283.89	170.74	169.75	20.20
	Female	4965	263.54	153.51	146.72	16.21
Rural	Both	2817	220.32	156.42	152.72	16.97
	Male	1535	234.67	167.03	166.52	19.26
	Female	1282	205.30	146.30	139.41	14.72

Age-specific morbidity

In 2015, the incidence of malignant tumors in Heilongjiang cancer registries increased slowly in patients less than 25 years of age and rapidly in those older than 25 years of age. Among those 15–50 years of age, the incidence of malignant tumors in women was higher than that in men. After 55 years of age, the incidence of malignant tumors in men was higher than that in women (Fig. 1).

Incidence of major cancers

In 2015, lung cancer had the highest overall incidence among tumor registries in Heilongjiang province (China), with a crude incidence of 60.32/100 000 and accounting for 23.21% of all new malignant tumor cases. This was followed by breast, liver, colorectal, thyroid, stomach, cervical, ovarian, corpus uteri, and pancreatic cancer. Lung cancer had the highest incidence in urban areas, with a crude incidence of 61.94/100 000, accounting for 22.65% of all new malignant tumor cases, followed by breast, colorectal, liver, thyroid, stomach, cervical, ovarian, prostate, and pancreatic cancers. Lung cancer also was the most common cancer in rural areas, with a crude incidence of 55.61/100 000, accounting for 25.24% of all new malignant tumor cases, followed by liver, breast, colorectal, stomach, cervical, thyroid, corpus uteri, ovarian, and pancreatic cancers (Table 2).

Mortality of malignant tumors

In 2015, the crude mortality rate of malignant tumors among Heilongjiang cancer registries was 164.69/100 000, with age-standardized mortality rates in Chinese (ASMRC) and in world (ASMRW) standard populations of 95.29/100 000 and 94.35/100 000 respectively, and cumulative rate (0–74 years) of 10.44%. The crude mortality rate of malignant tumors in urban areas was 169.51/100 000, with ASMRC and ASMRW of 92.20/100 000 and 91.71/100 000 respectively and cumulative rate (0–74 years) of 10.12%. In rural areas, the crude mortality rate of malignant tumors was 150.72/100 000, with ASMRC and ASMRW of 106.32/100 000 and 103.72/100 000 respectively, and cumulative rate (0–74 years) of 11.58% (Table 3).

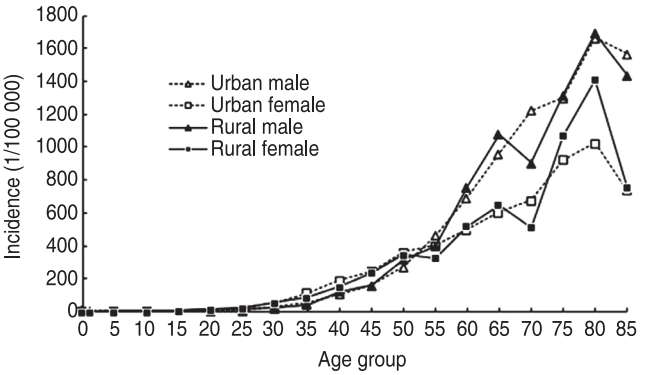

Fig. 1 Age-specific incidence of malignant tumors among cancer registries in Heilongjiang province, China

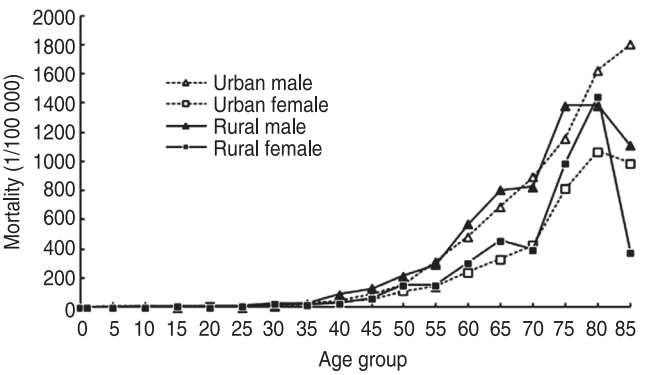

Fig. 2 Age-specific mortality of malignant tumors in cancer registries of Heilongjiang province, China

Table 2 Incidences of the top 10 types of cancer, Heilongjiang province, China, 2015

Rank	Both				Urban				Rural			
	Site	Incidence ($1/10^5$)	Proportion (%)	ASIRC ($1/10^5$)	Site	Incidence ($1/10^5$)	Proportion (%)	ASIRC ($1/10^5$)	Site	Incidence ($1/10^5$)	Proportion (%)	ASIRC ($1/10^5$)
1	Lung	60.32	23.21	34.64	Lung	61.94	22.65	33.75	Lung	55.61	25.24	38.29
2	Breast	47.04	9.15	29.75	Breast	53.56	9.99	32.76	Liver	35.27	16.01	24.95
3	Liver	27.44	10.56	16.31	Colorectum	30.16	11.03	16.77	Breast	27.38	6.11	19.40
4	Colorectum	26.74	10.29	15.57	Liver	24.74	9.04	13.87	Colorectum	16.82	7.63	11.50
5	Thyroid	18.39	7.08	13.73	Thyroid	20.34	7.44	15.14	Stomach	13.92	6.32	9.66
6	Stomach	17.55	6.75	10.15	Stomach	18.80	6.87	10.29	Cervix	13.29	2.95	9.74
7	Cervix	13.91	2.69	8.61	Cervix	14.12	2.62	8.27	Thyroid	12.75	5.79	9.80
8	Ovary	9.37	1.81	5.92	Ovary	9.77	1.81	5.92	Uterus corpus	11.85	2.63	8.27
9	Uterus corpus	9.09	1.76	5.74	Prostate	9.55	1.72	5.26	Ovary	8.17	1.81	5.98
10	Pancreas	8.47	3.26	4.80	Pancreas	8.66	3.17	4.66	Pancreas	7.90	3.59	5.35

Age-specific mortality

In 2015, the mortality of malignant tumors in the tumor registries of Heilongjiang province (China) increased slowly before the age of 30 and rapidly thereafter. The mortality among men was higher than that among women (Fig. 2).

Mortality of malignant tumors

Lung cancer ranked first among the malignant tumor deaths in the tumor registries of Heilongjiang province, with a crude mortality rate of 56.27/100 000 and accounting for 34.17% of all malignant tumor death cases. This was followed by liver, stomach, colorectal, breast, pancreatic, esophageal, ovarian, cervical, and prostate cancers. Lung cancer ranked highest among deaths due to malignant tumors in urban areas, with a crude mortality rate of 57.98/100 000, followed by liver, colorectal, gastric, breast, pancreatic, ovarian, esophageal, cervical, and prostate cancers. Lung cancer was the leading cause of cancer mortality in rural areas, with a crude mortality rate of 51.31/100 000, followed by liver, gastric, colorectal, breast, pancreatic, esophageal, cervical, ovarian, and brain cancers (Table 4).

Discussion

With the rapid development of science in recent years, we have gradually determined the underlying causes of cancer development and innovative cancer treatment schemes have gradually emerged; however, the burden of cancer is still relatively heavy [6]. From 2011 to 2015, the hospitalization medical expenses of cancer patients in China increased by 84.1%. In 2015, the expenses reached 177.1 billion yuan, accounting for 4.3% of total health expenses. Cancer has become the most important public health problem in China and currently faces significant challenges and economic burdens [7]. Therefore, promotion of the coverage of cancer registration in our province, building of a data platform for cancer, promotion of data exchange and sharing with cancer-related monitoring systems, and understanding of the dynamics of cancer incidence in our province will allow not only targeted prevention and control efforts but also provide a theoretical basis for cancer health prevention and control. In the 1980s, Heilongjiang province (China) initiated population-based cancer registries in the Nangang District of Harbin City, China. In 2009, with

Table 3 Cancer mortalities in Heilongjiang province, China, 2015

Areas	Gender	Deaths	Crued mortality (1/10^5)	ASMRC (1/10^5)	ASMRW (1/10^5)	Cumulative rate (0–74) (%)
Total	Both	8210	164.69	95.29	94.35	10.44
	Male	4994	201.64	123.03	122.85	14.03
	Female	3216	128.21	70.42	68.75	7.23
Urban	Both	6283	169.51	92.20	91.71	10.12
	Male	3777	207.24	120.18	120.47	13.76
	Female	2506	133.02	68.04	66.76	6.99
Rural	Both	1927	150.72	106.32	103.72	11.58
	Male	1217	186.05	132.02	130.37	14.97
	Female	710	113.70	80.37	76.89	8.15

Table 4 Mortality due to the top 10 types of cancer, Heilongjiang province, China, 2015

Rank	Both				Urban				Rural			
	Site	Mortality (1/10^5)	Proportion (%)	ASMRC (1/10^5)	Site	Mortality (1/10^5)	Proportion (%)	ASMRC (1/10^5)	Site	Mortality (1/10^5)	Proportion (%)	ASMRC (1/10^5)
1	Lung	56.27	34.17	36.92	Lung	57.98	34.20	30.94	Lung	51.31	34.04	35.86
2	Liver	25.20	15.30	16.49	Liver	23.18	13.67	12.85	Liver	31.05	20.60	21.96
3	Stomach	13.78	8.37	9.06	Colorectum	15.30	9.02	7.96	Stomach	12.28	8.15	8.54
4	Colorectum	13.60	8.26	8.91	Stomach	14.30	8.44	7.59	Colorectum	8.68	5.76	6.02
5	Breast	10.29	3.15	6.44	Breast	11.52	3.45	6.51	Breast	6.57	2.18	4.21
6	Pancreas	8.45	5.13	5.48	Pancreas	9.23	5.44	4.92	Pancreas	6.18	4.10	4.16
7	Esophagus	5.54	3.36	3.56	Ovary	5.84	1.75	3.36	Esophagus	6.02	4.00	4.06
8	Ovary	5.26	1.61	3.39	Esophagus	5.37	3.17	2.88	Cervix	4.80	1.56	3.56
9	Cervix	4.50	1.38	2.81	Cervix	4.41	1.32	2.36	Ovary	3.52	1.14	2.43
10	Prostate	3.15	0.95	2.28	Prostate	3.73	1.08	1.95	Brain, CNS	2.82	1.87	2.09

Note: CNS: central nervous system

support from central financial transfer payments, the coverage of cancer registration in Heilongjiang province has gradually expanded. The seven cancer registries included in the present study cover 13.12% of the total population of the province. Thus, the results reflect the current epidemic of malignant tumors in Heilongjiang province to a certain extent.

In 2015, the incidence of malignant tumors in the tumor registries in Heilongjiang province was 259.90/100 000. The ASIRW was 155.06/100 000, lower than the national average incidence (285.83/100 000) and ASIRW (186.39/100 000) in 2015 [8]. Compared to the 2014 data in this province (incidence of 263.62/100 000 and ASIRW of 156.41/100 000) [9], the incidence decreased slightly. Compared to 2014, ovarian cancer increased by one ranking while corpus uteri cancer decreased by one ranking, with the order of the other cancers remaining unchanged. The incidence of tumors in urban areas was higher than that in rural areas, with significant differences in the rankings of cancer types between urban and rural areas. In 2015, the mortality rate of malignant tumors among the tumor registries in Heilongjiang province was 164.69/100 000 with an ASMRW of 94.35/100 000, lower than the national average mortality rate and ASMRW in 2015 of 170.05/100 000 and 105.84/100 000, respectively. Compared to the data in 2014 (mortality 168.562/100 000 and ASMRW 100.972/100 000), the mortality rate was slightly reduced. The crude mortality rate in urban areas of Heilongjiang province was much higher than that in rural areas, while the standardized mortality rate was lower than that in rural areas, indicating that mortality rate of the elderly population in urban areas was higher while the adjusted mortality rate of the standard population was lower. Thus, the proportion of the elderly population in urban areas was larger than that in the standard population.

Lung, liver, and colorectal cancer are the most common malignant tumors in Heilongjiang province and are the focus of prevention and treatment efforts. In recent years, comprehensive prevention and treatment of cancer, early diagnosis and treatment of urban cancer, and early diagnosis and treatment of upper gastrointestinal cancer in rural areas have been taken up as key projects in our province. Standardization of early diagnosis and treatment of cancer and increased support for the early diagnosis and treatment of the key cancer species and high-risk groups are critical [10–11]; however, the high population base and increases in adverse behavioral, environmental, and dietary factors in recent years mean that there is still a long way to go in cancer prevention and control in this province [12–13].

Among the 31 provinces, cities, and autonomous regions in China, Heilongjiang province has the highest proportion of cancer deaths attributable to 23 carcinogenic factors (52.9%), while Shanghai has the lowest proportion (35.2%) [14]. External carcinogenic factors play an important role in the progress of cancer in Heilongjiang province. Therefore, we should formulate adaptive preventive and control measures according to the main carcinogenic factors. Effective control of these carcinogenic factors will significantly reduce the cancer burden in our province. Mortality due to breast, ovarian, and cervical cancer are among the top 10 and should be of concern to the administrative department of public health in our province. It is also an important task for future cancer prevention and treatment. Human papillomavirus (HPV) infection is a major risk factor for cervical cancer. Ovarian cancer is located in the pelvic cavity, a deep location in which this disease is not easy to find; thus, it is necessary to increase women's health care consciousness to develop good habits and customs; avoid unnecessary intake of exogenous estrogen; reduce intake of fatty food; educate women about breast cancer and prevention of tumors of the female reproductive system; and strive for early detection, diagnosis, and treatment [15–16].

In conclusion, the task of prevention and control of malignant tumors in Heilongjiang province is significant and poses new challenges. Realization of the goals of cancer prevention and control plans such as the Outline of the Health China 2030 Program requires the formulation of comprehensive cancer prevention and control strategies according to the current high incidence of cancer in Heilongjiang province. The network system for cancer prevention and control in this province should adopt health care, health education and health promotion, popularization of cancer prevention and treatment knowledge, improvement of unhealthy lifestyles, and exploration and development of opportunistic screening to reduce the cancer burden among residents.

Conflicts of interest

The authors indicate no potential conflicts of interest.

References

1. Mao F, Jiang YY, Dong WL, *et al*. On National Demonstration Areas: a cluster analysis. Chin J Epidemiol (Chinese), 2017, 38: 496–502.
2. Seneviratne K, Samaraweera S, Fernando E, *et al*. Strengthen cancer surveillance in Sri Lanka by implementing cancer registry informatics to enhance cancer registry data accuracy, completeness, and vimelines. Stud Health Technol Infom, 2017, 245: 1143–1147.
3. National Cancer Center. Guidelines for cancer registration in China (2016). Beijing: People's Health Publishing House, 2016. 59–75.
4. Bray F, Parkin DM. Evaluation of data quality in the cancer registry: principles and methods. Part I: comparability, validity and timeliness. Eur J Cancer, 2009, 45: 747–755.
5. Parkin DM, Bray F. Evaluation of data quality in the cancer registry: principles and methods. Part II. Completeness. Eur J Cancer, 2009,

45: 756–764.
6. Platz EA. Reducing cancer burden in the population: an overview of epidemiologic evidence to support policies, systems, and environmental changes. Epidemiol Rev, 2017, 39: 1–10.
7. Cai Y, Xue M, Chen W, *et al*. Expenditure of hospital care on cancer in China, from 2011 to 2015. Chin J Cancer Res, 2017, 29: 253–262.
8. Sun KX, Zheng RS, Zhang SW, *et al*. Report of cancer incidence and mortality in different areas of China, 2015. China Cancer (Chinese), 2019, 28: 1–11.
9. Sun HX, Chen WY, Zhang MX, *et al*. Analysis of cancer incidence and mortality in Heilongjiang cancer registries, 2014. Chin J Cancer Prevent Treat (Chinese), 2018, 25: 1407–1412.
10. Sun HX, Chen WY, Song BB. Analysis of cancer screening of 2013–2014 in Heilongjiang province. Pract Oncol J (Chinese), 2015, 29: 315–318.
11. Sun HX, Song BB, Chen WY, *et al*. Evaluation of upper gastrointestinal tract cancer early detection and early treated from 2009 to 2012 in Harbin, China. J Moder Oncol (Chinese), 2015, 23: 2518–2520.
12. Zeng H, Zheng R, Guo Y, *et al*. Cancer survival in China, 2003–2005: a population-based study. Int J Cancer, 2015, 136: 1921–1930.
13. Bao HL, Fang LW, Wang LH. The strategy for establishment of comprehensive cervical cancer prevention and control in the world. Chin J Prevent Med (Chinese), 2017, 51: 96–100.
14. Chen WQ, Xia CF, Zheng RS, *et al*. Disparities by province, age, and sex in site-specifc cancer burden attributable to 23 potentially modifable risk factors in China: a comparative risk assessment. Lancet Glob Health, 2019, 7: e257–e269.
15. Konecny GE. Securing global access to innovations in women's cancer care and control. Curr Opin Obstet Gynecol, 2016, 28: 1–3.
16. Zhang M, Liu ZZ, Zheng ZD, *et al*. Clinical observation of capecitabine monotherapy in elderly patients with advanced breast cancer. Oncol Transl Med, 2015, 1: 78–81.

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CASE REPORT

Primary gastric adenosquamous carcinoma: a case report and literature review

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Abstract

Primary gastric adenosquamous carcinoma (GASC) is exceedingly rare. It accounts for less than 1% of all gastric cancers. In this report, we describe our pathological findings along with a review of the literature to improve our understanding of the disease and reduce misdiagnosis, as well as to provide evidence for its treatment and prognosis. A 49-year-old male patient was admitted to our hospital (Dalian Municipal Central Hospital, Dalian, China) with a complaint of epigastric pain that had persisted for half a month. Physical examination, regular laboratory blood tests, and computed tomography revealed no obvious abnormalities. Gastroscopy revealed ulcers in the lower part of the stomach, and pathological assessment revealed adenocarcinoma. Radical gastrectomy was performed, and the folinic acid, fluorouracil, oxaliplatin (FOLFOX) chemotherapy regimen was administered postoperatively. Pathological assessment of the mass revealed a protruding tumor measuring 1.5 × 1.5 × 0.7 cm in the lower part of the stomach. The tumor infiltrated through the full wall of the stomach. This was confirmed by immunohistochemical (IHC) staining for cytokeratin (CK) (+), villin (-), p63 (++), and high-molecular-weight CK (+++). The patient remains alive with no recurrence more than seven years after surgery. Primary GASC is a rare malignant neoplasm. The diagnostic criteria for GASC mainly depend on the clinical, radiographic, and histopathological findings. Pathological assessment and IHC staining can be utilized to confirm the diagnosis. Radical gastrectomy plus postoperative chemotherapy containing the FOLFOX regimen is effective for treating GASC and might contribute to long-term survival.

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Key words: adenosquamous carcinoma; gastric cancer; treatment

Gastric cancer is one of the most common malignant tumors. Gastric adenocarcinoma is most prevalent in patients with gastric cancer, and squamous cell carcinoma is relatively rare with a poor prognosis [1]. Meanwhile, adenosquamous carcinoma is a rarer malignancy than adenocarcinoma and squamous cell carcinoma. Adenosquamous carcinoma contains elements of both squamous cell carcinoma and adenocarcinoma in the same tumor [1]. Primary gastric adenosquamous carcinoma (GASC) is characterized by the squamous cell carcinoma component making up ≥ 25% of the entire tumor mass [1–2]. GASC is an extremely rare entity and accounts for less than 1% of all gastric malignancies [3]; metastasis and recurrence commonly occur in the early stage, so the prognosis is poor. At present, the pathogenesis of GASC is still unclear, and its clinicopathological features and standardized treatment methods have not been established. Most publications regarding its clinicopathological manifestations are primarily in the form of case reports; therefore, there is limited awareness regarding GASC.

Here, we describe a patient with primary GASC with epigastric pain as the initial presentation who remains alive more than seven years after treatment that included

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a combination of surgery and chemotherapy to improve the diagnosis and treatment of GASC.

Case report

A 49-year-old male patient who presented with the chief complaint of epigastric pain that persisted for half a month was admitted to Dalian Municipal Central Hospital, China. The pain was paroxysmal, dull, and more serious after consuming a meal, but did not present with acid regurgitation, belching, nausea, or emesis. As the patient did not experience any relief from the pain after taking omeprazole orally, he visited our hospital on July 7, 2011. Physical examination showed a body temperature of 36.5℃, pulse rate of 60 beats/min, and blood pressure of 130/80 mmHg. His abdomen was soft and nontender, and the liver and spleen were not palpable. Laboratory data showed a hemoglobin level of 163 g/L and a hematocrit level of 43.9%. Chest and abdomen computed tomography did not identify any abnormalities in the liver, lungs, and kidneys. The levels of tumor markers, including carcinoembryonic antigen (CEA), alpha fetoprotein, and carbohydrate antigen 19-9 (CA19-9), were within normal limits.

Gastroscopy revealed an ulcerative lesion in the small curved side of the lower part of the stomach, about 1.2 × 1.2 cm in size, with rough and uneven surface mucosa, dirty coating, irregular surrounding mucosa, and unclear boundaries with surrounding tissues (Fig. 1). The pathological diagnosis of the biopsy specimen indicated adenocarcinoma. Laparoscopy-assisted total gastrectomy was performed successfully on July 11, 2011, and the patient recovered well. His postoperative hemoglobin level was 138 g/L, hematocrit level was 39.7%, and CEA and CA19-9 levels were 1.26 ng/mL and 2.77 U/mL, respectively. Pathological examination of the mass showed a protruding tumor measuring 1.5 × 1.5 × 0.7 cm, which infiltrated the full wall of the stomach, but had not invaded the incised edge. In addition, none of the perigastric lymph nodes showed metastasis, and vascular congestion, interstitial edema, and cancer invasion in the omentum were not detected (Fig. 2). The pathological manifestations under high-power lens confirmed the presence of a mixed-pattern carcinoma (glandular and squamous components). Immunohistochemical (IHC) staining analysis showed cytokeratin (CK) (+), villin (-), C-erbB2 (+), p63 (++), and high-molecular-weight CK (+++) (Fig. 3). Therefore, the pathological diagnosis of the resected specimens was well-differentiated GASC. The patient then received chemotherapy containing the folinic acid, fluorouracil, oxaliplatin (FOLFOX) regimen that included tegafur capsules, calcium folinate, and fluorouracil twice daily for two weeks with a one-week break, for a total of seven courses over five months. The patient recovered well, and his CEA and CA19-9 levels were 3.19 ng/mL and 4.48 U/mL, respectively, after chemotherapy. The patient is currently alive and has had no recurrence.

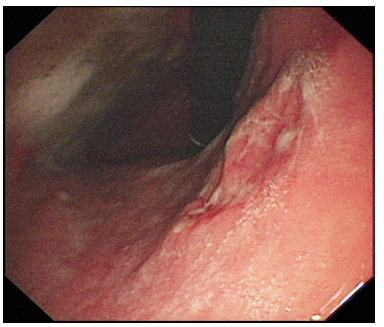

Fig. 1 Gastroscopy showing a sunken ulcerative lesion (1.2 × 1.2 cm) in the small curved side of the lower part of the stomach

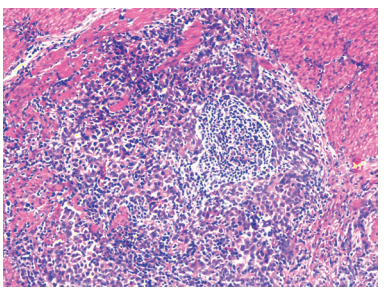

Fig. 2 There was infiltration of poorly differentiated squamous cell carcinoma with slight adenocarcinoma components (hematoxylin and eosin staining, × 100)

Discussion

Adenosquamous carcinoma has been reported to develop in various sites in the digestive tract, such as the esophagus, esophagogastric junction, colon, and hepatobiliary tract; it has aggressive clinicopathological features and a poor prognosis. GASC is a rare cell type of gastric cancer compared with the predominant adenocarcinoma type that has an incidence of more than 90% [4]. Differences in etiology, pathogenesis, and survival also exist between gastric adenocarcinoma and GASC. Some authors have reported that GASC is a mixed neoplasia (gland-like and squamous) and has a male:female ratio of approximately 4:1 [5-8]. The histogenesis of GASC is still unclear. Several hypotheses have been postulated on the origin and metaplastic transformation of GASC [5, 7, 9–10], such as squamous metaplastic transformation of adenocarcinoma, cancerization of ectopic squamous epithelium [6–7], collision of adenocarcinoma and squamous cell carcinoma [7], cancerization of metaplastic squamous cells [9], and stem cell differentiation toward

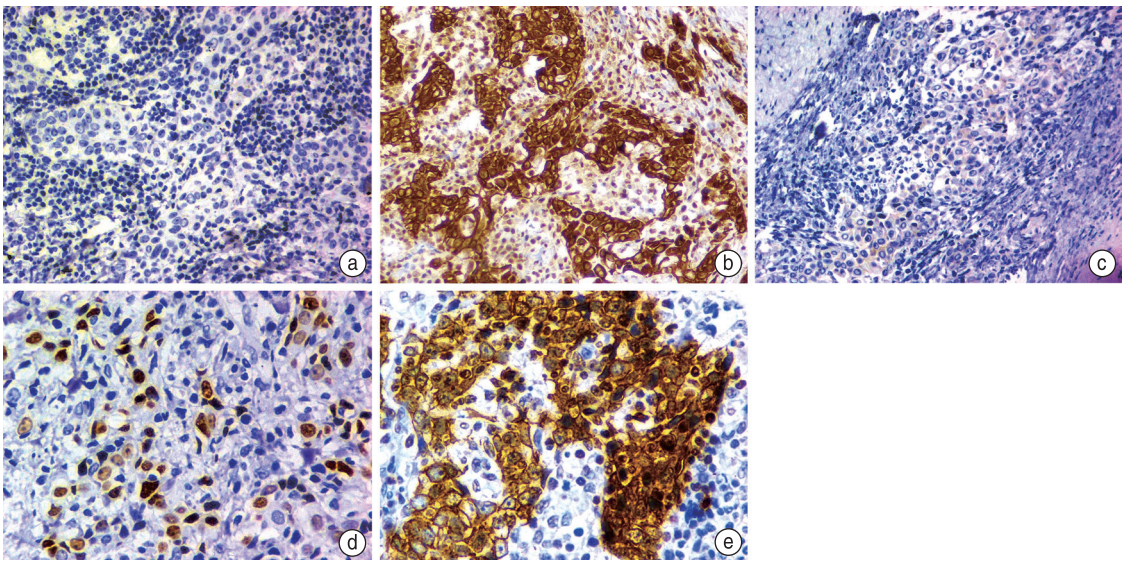

Fig. 3 IHC analysis. The neoplastic cells tested negative for villin (a) and positive for CK (b), C-erB2 (c), P63 (d), and high-molecular-weight cytokeratin (e)

two cell lines: glandular and squamous [8, 10].

The clinical manifestations of GASC are not consistent and similar with those of gastric adenocarcinoma; it is mainly manifested as abdominal pain, and a few patients manifest acid regurgitation, abdominal distension, and black stool. In the present case, the patient was a middle-aged man; the first clinical manifestation was epigastric pain; there were no other positive pathological manifestations; and laboratory blood tests, tumor markers, and computed tomography showed no obvious abnormalities. Gastroscopy revealed ulcer lesions in the lower part of the stomach.

In the diagnosis of GASC, endoscopic biopsies are superficial and limited in scope, whereas the differentiation, distribution, and composition of squamous cell carcinoma and adenocarcinoma are usually diversified. Preoperative diagnosis of GASC is particularly difficult, and most patients can only be diagnosed by relying on postoperative pathology and IHC analysis. The IHC features of GASC are squamous epithelial CK5/6 expression, CK macromolecule, obvious p63 positivity, focal CK8/18 expression, glandular epithelial low-molecular-weight CK expression, and obvious CK8/18 expression [11]. Chen *et al* have pointed out that poorly differentiated squamous cell carcinoma in gastric adenosquamous carcinoma is not easy to distinguish from neuroendocrine carcinoma, which could be examined by positive expression of chromogranin and synaptophysin [12]. In our case, there was CK macromolecule and obvious p63 positivity in squamous cell carcinoma components but negativity in adenocarcinoma components. CK was weakly positive in adenocarcinoma components, supporting the diagnosis of GASC. Therefore, to improve the accuracy of diagnosis, when GASC is morphologically suspected, additional IHC staining of CK, CK5/6, P63, CK8/18, and CK macromolecule should be performed.

A standard treatment approach for GASC has yet to be established. Radical surgery is extremely crucial when feasible. Similarly, adjuvant therapy such as chemotherapy, immunotherapy, and radiotherapy are also important in prolonging survival and improving the quality of life of patients postoperatively. Ebi *et al* [7] reported that postoperative S-1 chemotherapy may therefore be very useful for treating patients with GASC and that peritoneal metastasis is one of the main factors for the dismal prognosis. The 5-year survival rate is approximately 10%, and the median survival time is only 12 months. A case of palliative gastrectomy for a GASC with peritoneal dissemination in a patient who underwent a course of systemic chemotherapy containing S-1 plus paclitaxel (PTX) after surgery was reported by Hirano *et al* [13]. No serious adverse events were observed, and treatment with S-1 plus PTX was continued for 1 year before being switched to adjuvant chemotherapy containing S-1 alone for another year. The patient remained in clinical remission and survived for over 8 years. In our case, the patient underwent radical total gastrectomy, was followed up for seven courses of chemotherapy containing the FOLFOX regimen, and survived without recurrence after seven years of follow-up.

GASC is characterized by more aggressive clinicopathological features and has a poor prognosis despite its major biological determinant being the adenocarcinoma component [14–15]. Feng *et al* reported that most patients with GASC developed lymph node

metastasis and that most patients were diagnosed in the middle and late stages [16]. Li *et al* summarized the clinical data of 42 cases of primary GASC. They found that all patients had an average survival time of 36.4 months; median survival time of 28.0 months; and overall 1-, 3-, and 5-year survival rates of 82.2%, 42.3%, and 18.2%, respectively [17]. Univariate analysis revealed that tumor size, Borrmann type, tumor differentiation, radical gastrectomy, lymph node metastasis, and clinical stage were associated with postoperative survival. Multivariate analysis revealed that tumor differentiation, radical gastrectomy, and clinical stage were independent prognosis factors. In our case, the patient underwent radical total gastrectomy, was followed up with chemotherapy containing FOLFOX, and survived for more than seven years.

In conclusion, a rare case of GASC that was successfully controlled with surgery and chemotherapy was herein described. Postoperative FOLFOX chemotherapy may therefore be very useful for treating patients with GASC. Due to the limited number of cases we collected, the long-term therapeutic effects need to be further studied.

Conflicts of interest

The authors indicate no potential conflicts of interest.

References

1. Zhang N, Deng J. Research progress of the clinically uncommon gastric carcinoma. Chin J Gastroinbtest Surg (Chinese), 2018, 21: 228–235.
2. Chen H, Shen C, Yin R, *et al*. Clinicopathological characteristics, diagnosis, treatment, and outcomes of primary gastric adenosquamous carcinoma. World J Surg Oncol, 2015, 13: 136.
3. Faria GR, Eloy C, Preto JR, *et al*. Primary gastric adenosquamous carcinoma in a Caucasian woman: a case report. J Med Case Rep, 2010, 4: 351.
4. Crew KD, Neugut AI. Epidemiology of gastric cancer. World J Gastroenterol, 2006, 12: 354–362.
5. Mori M, Iwashita A, Enjoji M. Squamous cell carcinoma of the stomach: report of three cases. Am J Gastroenterol, 1986, 81: 339–342.
6. Boswell JT, Helwig EB. Squamous cell carcinoma and adenoacanthoma of the stomach. Cancer, 1965, 18: 181–192.
7. Ebi M, Shimura T, Yamada S, *et al*. A patient with gastric adenosquamous carcinoma with intraperitoneal free cancer cells who remained recurrence-free with postoperative S-1 chemotherapy. Intern Med, 2012, 51: 3125–3129.
8. Straus R, Heschel S, Fortmann DJ. Primary adenosquamous carcinoma of the stomach A case report and review. Cancer, 1969, 24: 985-95.
9. Blazquez S, Raventos A, Diaz ML, *et al*. Adenosquamous gastric carcinoma in Caucasian patient. Rev Esp Enferm Dig, 2005, 97: 211–212.
10. Mingazzini PL, Barsotti P, Malchiodi Albedi F. Adenosquamous carcinoma of the stomach: histological, histochemical and ultrastructural observations. Histopathology, 1983, 7: 433–443.
11. Shen J, Lu H. Primary adenosquamous carcincoma of stomach: A report of two cases with review of literature. J Huai hai Med, 2013, 31: 488–489.
12. Chen C, Zheng X, Pan W, *et al*. Clinical Pathologr and Ultrastructural Observations on 5 case of Gastric Adenosquamous Carcinoma. J Shanghai Med Univ (Chinese), 1987: 417–420.
13. Hirano M, Ozamoto Y, Ichinose M, *et al*. A case of gastric adenosquamous carcinoma with peritoneal dissemination in which treatment with S-1 plus paclitaxel therapy resulted in improved long-term survival. Gan To Kagaku Ryoho, 2014, 41: 885–888.
14. Song B, Liu X, Ma H, *et al*. A clinical series of 80 patients with adenosquamous carcinoma of pancreas. Chin J Surg (Chinese), 2014, 52: 658–661.
15. Bansal RK, Sharma P, Kaur R, *et al*. Primary gastric adenosquamous carcinoma in an Indian male. Indian J Pathol Microbiol, 2013, 56: 416–418.
16. Feng F, Zheng G, Qi J, *et al*. Clinicopathological features and prognosis of gastric adenosquamous carcinoma. Sci Rep, 2017, 7: 4597.
17. Li B, Sun L, Wang X, *et al*. Analysis of clinicopathological characteristics and prognosis on 42 patients with primary gastric adenosquamous cell carcinoma. Chin J Gastroinbtest Surg (Chinese), 2017, 20: 207–212.

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Condylar invasion after postoperative chemotherapy for primary intraosseous squamous cell carcinoma of the mandible: a case report

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Abstract

Primary intraosseous squamous cell carcinoma (PIOSCC) is a rare malignant tumor that occurs predominantly in the jaw. Pathological evidence shows that PIOSCC originates from the residual odontogenic epithelium and a preexisting odontogenic cyst or tumor. Here, we report the case of a 63-year-old man with central squamous cell carcinoma of the mandible. On the basis of imaging and histopathology reports, the patient was diagnosed with PIOSCC of the jaw. Subsequently, he was treated with postoperative adjuvant chemotherapy. During a postoperative follow-up visit in the ninth month, mandibular computed tomography (CT) scanning and bone imaging revealed local recurrence and condylar invasion. Therefore, radiation along with chemotherapy was administered. This case study adds to the literature on PIOSCC and widens the understanding of its diagnosis, treatment, and prognosis.

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Key words: PIOSCC; condyle invasion; postoperative chemotherapy

Primary intraosseous squamous cell carcinoma (PIOSCC) of the mandible is a rare malignant intramaxillofacial tumor. There are few reported cases; therefore, epidemiology, treatment, and prognosis of the disease remain unclear. Here we report a case of PIOSCC of the mandible [1–2].

Case presentation

In May 2017, a 63-year-old man with odontalgia on the left lower posterior tooth underwent exodontia at the Department of Oral Surgery, Hospital of Yilong County. The toothache persisted post operation; this was accompanied by a non-healing extraction socket and an emerging feeling of numbness in the left lower lip. The patient was later referred to the Department of Oral and Maxillofacial Surgery, West China Hospital of Sichuan University, for review of the above symptoms. Upon physical examination, the patient was found to have a lump on the left side of the face with swelling around the angle of the mandible; 37 teeth were missing and a cauliflower-like hard ulcer with unclear margins was observed. A hard, mobile, cervical lymph node measuring 1 centimeter in diameter was identified through palpation on the right side of the neck. As there was no contraindication, surgery was performed on August 2017. The patient was sedated under general anesthesia; the operative procedure involved resection of the left mandibular mass plus left mandibulectomy (half of the mandible), gum resection, cervical lymph node dissection plus left laryngeal recurrent nerve surgical exploration plus left carotid artery and right femoral anterolateral flap vascularized free graft. Postoperative pathological diagnosis showed grade I PIOSCC in the left mandible (Fig. 1). Cervical lymph nodes showed normal histoarchitecture. Subsequently, the patient received two cycles of chemotherapy (a combination of tegafur and lobaplatin). Bone imaging of condylar invagination after postoperative chemotherapy revealed no local recurrence.

In May 2018, on the ninth month during a postoperative follow-up visit, the patient noticed a recurrence of the left buccal swelling and experienced pain. Computed

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tomography (CT) scan and bone imaging of the mandible (Fig. 2) revealed local recurrence and condylar invasion. The patient received two cycles of chemotherapy (a combination of docetaxel, cisplatin, and tegafur). He was then referred to the Department of Oncology, Affiliated Hospital of North Sichuan Medical College, for further treatment. When the patient presented at our facility, the left face was swollen, and the patient was experiencing local pain (Fig. 3). Panoramic radiograph and CT (Fig. 4, 5) scan showed the invasion of the condyle after postoperative chemotherapy. The patient then received local radiotherapy (radiotherapy planning: first stage: p-gtv 44Gy/20Fx (95%); second stage: p-Gtv 26.4 Gy/12Fx (95%), and concurrent sensibilization chemotherapy based on endostar.

Discussion

PIOSSC is a rare malignant tumor of the mandible. It commonly originates from the residual cells of the enamel epithelium of the tooth germ and a preexisting odontogenic cyst or tumor [3]. PIOSSC commonly affects men, with a high incidence observed in men aged > 50 years. The mandible is primarily affected by bone cancer, suggesting an origin from epithelial cells that exist within the bone. These epithelial cells can proliferate and give rise to odontogenic carcinoma. Often, this process is triggered by inflammation [4–5]. In this case, we do not know whether the patient had an odontogenic jaw cyst in the past; however, the patient reported having dead teeth. Early detection of an odontogenic jaw cyst and the presence of chronic inflammatory signs should be followed by prompt treatment, which can reduce the extent of malignancy originating from a benign odontogenic cyst.

In this case, distant metastasis was not identified during the initial diagnosis. A CT scan suggested bone destruction and expansion of the medulla to the cortex, suggestive of PIOSCC of the mandible. The condylar process was spared in the operation of this patient and the bone flap fashioned. Although occlusion remained unaffected, condylar invasion occurred during postoperative adjuvant chemotherapy; this indicated a high degree of recurrence, in addition to the aggressive nature of the tumor leading to local invasion. The standard treatment of PIOSCC involves combining chemotherapy and postoperative

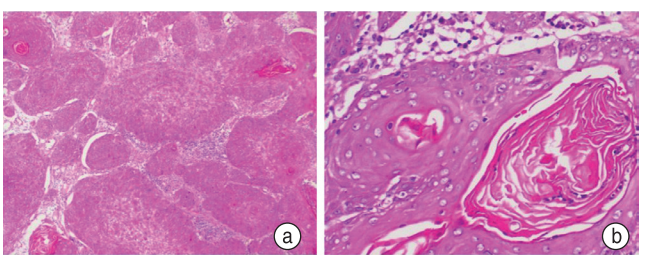

Fig. 1 Postoperative pathology of the mandibular mass. (a) HE staining × 400; (b) HE staining × 40

Fig. 2 Bone imaging of condylar invagination after postoperative chemotherapy

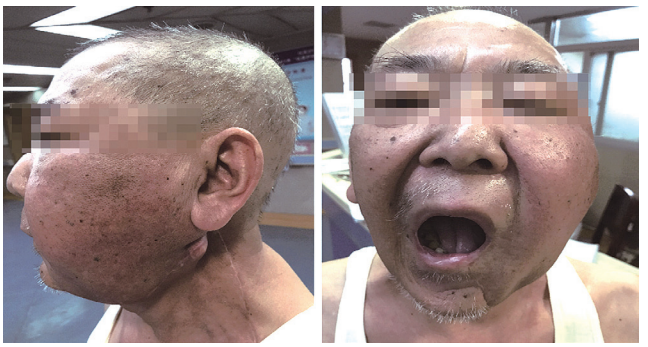

Fig. 3 Buccal asymmetry was observed on the left side of the patient's face

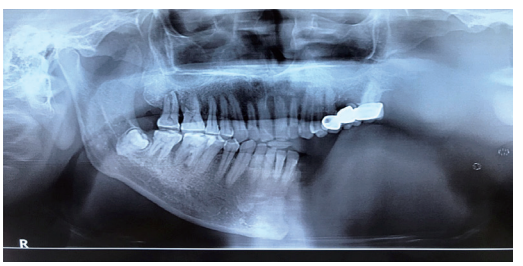

Fig. 4 Panoramic radiograph shows the missing left mandible

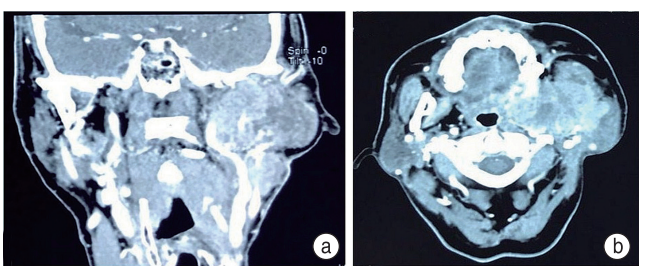

Fig. 5 CT scans of condylar invasion after postoperative chemotherapy. a: sagittal section; b: coronal section

radiotherapy [5-7]. Complete resection is performed based on the prognosis before extensive involvement of adjacent tissues or lymph node metastases; early aggressive surgical treatment helps to reduce local recurrence [8].

PIOSCC of the mandible has a short course and a rapid replication rate, and it is often found in the body and ascending ramus of the mandible. The early symptoms are not obvious. When the tumor encroaches on the alveolar nerve, toothache, tooth loosening, a non-healing wound after tooth extraction, and lower lip numbness occur [7, 9]. In this case, the patient experienced toothache, for which he underwent tooth extraction. The patient's condition then gradually deteriorated, and he presented with lower lip numbness and jaw pain. This indicated a high probability of misdiagnosis. Studies have shown that the prognosis of PIOSCC is related to the status of positive lymph nodes and histological grade of the tumor [10–11]. This patient did not receive radiotherapy postoperatively but received local radiotherapy after local invasion. There is no consensus on whether concurrent chemoradiation or preoperative induction chemotherapy followed by local radiotherapy is better.

In conclusion, early diagnosis of PIOSCC is extremely important. For middle-aged men with toothache, facial numbness, and no remission of symptoms after tooth extraction, CT and biopsy should be performed at initial presentation. The treatment of this disease primarily involves extensive surgical excision of the tumor. In addition, neck dissection could be performed in combination with postoperative radiotherapy and chemotherapy for patients who are suspected to have lymph node metastasis. Postoperative chemoradiation may be more effective than chemoradiation alone. This may improve the survival rate of patients.

Conflicts of interest

The authors declare no potential conflicts of interest.

References

1. Woolgar JA, Triantafyllou A, Ferlito A, *et al*. Intraosseous carcinoma of the jaws: a clinicopathologic review. Part III: Primary intraosseous squamous cell carcinoma. Head Neck, 2013, 35: 906–909.
2. Woolgar JA, Triantafyllou A, Ferlito A, *et al*. Intraosseous carcinoma of the jaws – a clinicopathologic review. Part I: Metastatic and salivary-type carcinomas. Head Neck, 2013, 35: 895–901.
3. Kikuchi K, Ide F, Takizawa S, *et al*. Initial-stage primary intraosseous squamous cell carcinoma derived from odontogenic keratocyst with unusual keratoameloblastomatous change of the maxilla: a case report and literature discussion. Case Rep Otolaryngol, 2018: 7959230.
4. Bodner L, Manor E, Shear M, Shear M, *et al*. Primary intraosseous squamous cell carcinoma arising in an odontogenic cyst: a clinicopathologic analysis of 116 reported cases. J Oral Pathol Med, 2011, 40: 733–738.
5. Pardhe N, Bhagalia S, Nayak PA, *et al*. Primary intraosseous squamous cell carcinoma: a devil in disguise. BMJ Case Rep, 2013, pii: bcr2013009639.
6. Bessell A, Glenny AM, Furness S, *et al*. Interventions for the treatment of oral and oropharyngeal cancers: surgical treatment. Cochrane Database Syst Rev, 2011, CD006205
7. Alotaibi O, Al-Zaher N, Alotaibi F, *et al*. Solid-type primary intraosseous squamous-cell carcinoma in the mandible: Report of a rare case. Hematol Oncol Stem Cell Ther, 2016, 9: 118–122.
8. Suei Y, Tanimoto K, Taguchi A, *et al*. Primary intraosseous carcinoma: review of the literature and diagnostic criteria. J Oral Maxillofac Surg, 1994, 52: 580–583.
9. Yamada T, Ueno T, Moritani N, *et al*. Primary intraosseous squamous cell carcinomas: five new clinicopathologic case studies. J Craniomaxillofac Surg, 2009, 37: 448–453.
10. Wenguang X, Hao S, Xiaofeng Q, *et al*. Prognostic factors of primary intraosseous squamous cell carcinoma (PIOSCC): a retrospective review. PLoS One, 2016, 11: e0153646.
11. Huang JW, Luo HY, Li Q, *et al*. Primary intraosseous squamous cell carcinoma of the jaws. Clinicopathologic presentation and prognostic factors. Arch Pathol Lab Med, 2009, 133: 1834–1840.

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