

Cancer stem cell markers correlate with early recurrence and survival in hepatocellular carcinoma

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Abstract

AIM: To investigate whether expression of cancer stem cell (CSC) markers is associated with recurrence and survival in hepatocellular carcinoma (HCC) patients.

METHODS: A consecutive series of 90 HCC patients who underwent curative hepatectomy between April 2007 and April 2009 were analyzed. Of the 90 patients, 38 (42%) experienced recurrence within two years of surgery. To adjust for baseline differences between this early recurrence group and the other patients, propen-

sity-score matching was used to generate 25 pairs of patients. Immunohistochemistry was used to compare expression of CD133, CD90, and epithelial cell adhesion molecule (EpCAM) in liver tissues from propensity score-matched patients and from 10 healthy adults. Associations of the three markers with HCC, clinicopathological characteristics, early recurrence, and survival time were explored.

RESULTS: The expression of all three CSC markers was significantly higher in HCC tissue than in healthy liver tissue ($P < 0.001$ for all). Among the HCC clinicopathology characteristics examined, the absence of tumor capsule was associated with CD133 expression ($P = 0.005$); higher histopathology grade and larger tumor size were associated with CD90 expression ($P = 0.010$ and 0.034 , respectively); and elevated serum alpha-fetoprotein levels were associated with EpCAM expression ($P = 0.021$). Expression of CD90 and EpCAM was significantly higher in the early recurrence group than in other patients ($P = 0.001$ and 0.045 , respectively), whereas CD133 expression was not significantly different between the two groups ($P = 0.440$). Multivariate analysis identified only CD90 expression as significantly associated with early recurrence. Log-rank analysis identified expression of both CD90 and EpCAM as significantly associated with survival time of HCC patients. Cox regression identified EpCAM expression as an independent predictor of survival time.

CONCLUSION: Expression of CD133, CD90, and EpCAM CSC markers may be linked to HCC tumor onset and/or progression. In addition, EpCAM expression is associated with shorter survival time, while CD90 expression is associated with early HCC recurrence.

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Key words: Hepatocellular carcinoma; Cancer stem cells; CD133; CD90; Epithelial cell adhesion molecule

Core tip: Cancer stem cells have been proposed as the cells responsible for initiating tumor formation, recurrence and metastasis, and liver cancer stem cells have been found to carry the surface markers CD133, CD90, and epithelial cell adhesion molecule (EpCAM). This paper addresses the clinical impact of CD133, CD90, and EpCAM in propensity score-matched patients with hepatocellular carcinoma. Our findings revealed that expression of CD133, CD90, and EpCAM may be linked to hepatocellular carcinoma (HCC) tumor onset and/or progression. In addition, EpCAM expression is associated with shorter survival time, while CD90 expression is associated with early HCC recurrence.

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INTRODUCTION

Hepatocellular carcinoma (HCC) is one of the most common cancers worldwide, with nearly one million new cases diagnosed every year around the world^[1]. Hepatic resection remains the most effective and practical treatment for HCC patients^[2,3]; single-site studies indicate that this method can achieve five-year survival rates as high as 50%^[4,5]. However, postoperative recurrence, which can be as high as 45% within two years of surgery^[6], and the associated poor prognosis remain challenges for HCC management^[7,8].

The cancer stem cell (CSC) hypothesis stipulates that primary tumors are initiated and maintained by a small population of cancer cells with “stem cell-like” characteristics^[9]. In support of this hypothesis, CSCs have been identified in many tumor types, including HCC^[10]. Numerous cell surface antigens, such as CD133, CD90, and epithelial cell adhesion molecule (EpCAM) have been identified as CSC markers, and all three are expressed by liver CSCs (LCSCs) in HCC. Such markers may prove useful for predicting the prognosis of HCC patients, since the CSC hypothesis predicts that CSCs drive tumor recurrence and metastasis after hepatic resection^[9]. Therefore we aimed to analyze the expression of CD133, CD90, and EpCAM in patients with HCC and to search for associations with early recurrence and survival time.

MATERIALS AND METHODS

This study was approved by the ethics committee of the Tumor Hospital, Guangxi Medical University, and written informed consent was obtained from participants prior to enrollment.

Patients and healthy controls

A consecutive sample of 90 HCC patients treated by curative hepatectomy at our hospital between April 2007 and April 2009 was enrolled in our study. To be enrolled, patients had to have pathology-confirmed HCC that had not been treated with any other anticancer modality, and their hepatectomy had to be confirmed as curative based on the following criteria: (1) the surgery was limited to a solitary nodular tumor; (2) the resection margin was greater than 10 mm; (3) post-surgical imaging did not show residual tumor^[11], extrahepatic metastases or portal tumor thromboses^[12]; and (4) levels of alpha-fetoprotein (AFP) in patients with elevated serum AFP levels before surgery decreased to normal within two months after the procedure. Patients with multiple tumors were excluded, such as those with macroscopic intrahepatic metastases adjacent to the primary tumor, or those with extrahepatic metastases. Liver samples from 10 adult patients treated surgically for hepatic injury or hemangioma were collected as controls.

Propensity-score matching based on early recurrence

Since clinicopathological characteristics related to tumor recurrence have been shown to cause significant baseline differences in cancer patients, which can bias subsequent analyses^[13], we used propensity-score matching^[14] to generate pairs in which one patient experienced recurrence within two years of hepatectomy and the other did not. These pairs were generated using one-to-one matching without replacement and a 0.2 caliper width^[15].

Follow-up

All patients were followed one month after hepatectomy, then every three months during the first year after surgery, and every six months thereafter. During follow-up visits, patients were subjected to a physical examination, liver function tests, assay of serum AFP, abdominal ultrasonography, and computed tomography (CT) or magnetic resonance imaging (MRI) of the liver. Patients were diagnosed with recurrence when ultrasonography, dynamic CT or MRI detected a new hepatic lesion. The end-point for follow-up was defined as three years, and the survival time was defined as 36 mo for those who survived more than three years.

Immunohistochemistry of CSC markers

Surgical tissues were fixed in 10% formalin, embedded in paraffin, cut into 4- μ m sections, deparaffinized in xylene and rehydrated through graded alcohol solutions. Antigen retrieval was performed for 5 min at 100 °C in citrate buffer (10 mmol/L, pH 6.0) in a microwave oven. Endogenous peroxidases were blocked by immersing the sections in 3% hydrogen peroxide for 15 min. Sections were then incubated at 37 °C for one hour with a rabbit monoclonal antibody against human CD133 (1:100; Miltenyi, CA, United States), a mouse monoclonal antibody against human CD90 (1:100; Eptomics, CA,

Table 1 Baseline characteristics

Characteristic	ER group <i>n</i> = 38	NER group <i>n</i> = 52	<i>P</i> value
Age (yr)	48.4 ± 10.6	47.2 ± 12.9	0.637
BMI (kg/m ²)			
< 23	28	34	0.401
≥ 23	10	18	
Total bilirubin (mg/dL)	14.0 ± 6.3	13.3 ± 5.7	0.585
Albumin (g/L)	40.0 ± 3.9	41.0 ± 4.9	0.300
ALT (U/L)	38.1 ± 31.5	40.2 ± 39.7	0.767
AST (U/L)	51.9 ± 40.0	47.3 ± 46.1	0.850
Prothrombin time (s)	13.0 ± 2.1	12.6 ± 1.3	0.346
Platelet count (10 ⁹ /L)	150.2 ± 49.0	196.7 ± 83.5	0.003
AFP (ng/mL)			
< 400	24	37	0.423
≥ 400	14	15	
Tumor size (cm)	7.0 ± 3.0	6.0 ± 2.9	0.093
Liver cirrhosis			
Present	26	22	0.014
Absent	12	30	
Tumor capsule			
Present	23	32	0.038
Absent	15	20	
Edmondson grade			
I or II	17	28	0.393
III or IV	21	24	

Baseline characteristics of hepatocellular carcinoma (HCC) patients who experienced recurrence (ER) within two years of hepatectomy (ER group) and HCC patients who did not (NER group). AFP: Alpha-fetoprotein; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; BMI: Body mass index.

United States), or a mouse monoclonal antibody against human EpCAM (1: 100; Eptomics). Sections were rinsed with phosphate-buffered saline (PBS), incubated with biotinylated anti-rabbit or anti-mouse immunoglobulin diluted in PBS for 20 min at room temperature, and rinsed again with PBS. Sections were incubated with anti-horseradish peroxidase conjugate for 10 min, rinsed in PBS, and incubated with diaminobenzidine for 10 min. Finally, sections were counterstained with hematoxylin. Negative controls were prepared in the same way except that they were incubated with PBS instead of primary antibodies.

Stained sections were examined by two experienced hepatopathologists (Ou C, Zeng LX) who were blinded to the clinicopathological data of the tissue samples. To assess CD133, CD90, and EpCAM expression, the numbers of cells positive for these markers were counted in five non-overlapping, randomly selected × 400 fields containing a total of at least 1000 cells. Expression levels in each patient or control were quantified as the percentage of the total number of cells in the fields that were positive for CD133, CD90, or EpCAM. For statistical analysis, patients were categorized as negative for these markers if the percentage of CD133, CD90, or EpCAM-positive cells was below 5%, or positive if the percentage was 5% or greater.

Statistical analysis

All statistical analyses were performed using SPSS 19.0

(IBM, United States). Results were reported as averages or relative risk (RR) ratios with 95% CIs. The chi-squared test was used to compare categorical data, while the *t* test was used to compare continuous data. Survival curves were constructed using the Kaplan-Meier method, and differences between curves were analyzed using the log-rank test. Multivariate Cox proportional hazard regression was used to assess the ability of variables to predict overall survival. All statistical tests were two-sided, with the threshold for significance defined as *P* < 0.05.

RESULTS

During the study period, 729 HCC patients were treated at our hospital and 215 (29.5%) were excluded because they had been treated initially for HCC at other centers. Among the remaining 514 patients, 157 (30.5%) had solitary nodular tumors without extrahepatic metastases or portal tumor thromboses. We excluded 55 of these (35.0%) because they had received only local ablation therapy, ethanol injection or transarterial chemoembolization, and we excluded another 12 (7.6%) because they did not participate in follow-up. The remaining 90 (57.3%) patients satisfied our inclusion criteria and were included in our study. Of these, 38 (42%) experienced intrahepatic recurrence within two years after curative hepatectomy; these patients were assigned to the early recurrence (ER) group. The remaining 52 patients did not experience recurrence within two years and were therefore assigned to the non-ER (NER) group. Table 1 summarizes the demographic and clinicopathological characteristics of both groups. To provide a comparison with HCC patients, we also collected liver tissue samples from 10 healthy adults who had undergone surgery for non-HCC problems.

The NER group showed a significantly higher frequency of tumor capsule and significantly elevated platelet count than did the ER group. However, the ER group showed a significantly higher frequency of liver cirrhosis. The two groups did not differ significantly in age, body mass index (BMI), serum bilirubin, albumin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), prothrombin time, AFP, tumor size or Edmondson grade.

To reduce confounding due to covariates related to cancer recurrence, previously observed in studies of HCC^[13,16], we used propensity-score matching to generate 25 pairs of ER and NER patients. No significant baseline differences were observed between these pairs (Table 2), which were then used in subsequent analysis.

Association of CD133, CD90, and EpCAM expression with HCC

Of the 50 tumor samples examined, 44 (88%) showed CD133 expression by immunohistochemistry, with each sample showing an average of 37.7% ± 26.0% of CD133-positive cells. A total of 42 samples (84%) were

Table 2 Baseline characteristics of experienced recurrence and non-experienced recurrence patients with hepatocellular carcinoma, after propensity-score matching

Characteristic	ER group <i>n</i> = 25	NER group <i>n</i> = 25	<i>P</i> value
Age (yr)	46.6 ± 8.9	45.7 ± 13.7	0.784
BMI (kg/m ²)			
< 23	18	16	0.544
≥ 23	7	9	
Total bilirubin (mg/dL)	13.2 ± 2.5	15.4 ± 6.4	0.989
Albumin (g/L)	39.3 ± 3.5	40.3 ± 4.7	0.371
ALT (U/L)	39.6 ± 16.9	34.6 ± 12.4	0.232
AST (U/L)	50.4 ± 35.0	47.8 ± 24.9	0.767
Prothrombin time (s)	13.2 ± 2.5	12.7 ± 1.6	0.466
Platelet count (10 ⁹ /L)	141.5 ± 42.0	157.4 ± 64.6	0.308
AFP (ng/mL)			
< 400	17	20	0.333
≥ 400	8	5	
Tumor size (cm)	6.9 ± 3.0	6.0 ± 2.9	0.164
Liver cirrhosis			
Present	18	18	1.000
Absent	7	7	
Tumor capsule			
Present	7	7	1.000
Absent	18	18	
Edmondson grade			
I or II	8	12	0.248
III or IV	17	13	

AFP: Alpha-fetoprotein; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; BMI: Body mass index; ER: Early recurrence; NER: Non-ER.

categorized as CD133-positive because the percentage of CD133-positive cells was at least 5%. The protein was present mainly in the cytoplasm (Figure 1A). CD90 expression was observed in 47 samples (94%), with each sample showing an average of 5.5% ± 2.9% of CD90-positive cells. However, only 32 samples (64%) were categorized as CD90-positive. The protein was observed exclusively in the cytoplasm (Figure 1B). EpCAM expression was detected in 39 samples (78%), with each sample containing an average of 4.6% ± 3.8% of EpCAM-positive cells. Only 21 samples (42%) were categorized as EpCAM-positive. The protein was observed mostly on the membrane of tumor cells (Figure 1C).

In contrast to these results with CSC markers in tumor tissues, none of the 10 normal adult liver samples showed detectable expression of CD133, CD90, or EpCAM under the same antibody staining conditions used with the tumor samples (data not shown; *P* < 0.001 for all).

To explore whether expression of CD133, CD90, or EpCAM may correlate with HCC oncogenesis, we examined potential associations of CSC marker expression with the following dichotomized HCC clinicopathological variables: age, < 50 or ≥ 50 years; BMI, < 23 or ≥ 23 kg/m²; serum bilirubin, ≤ 17.1 or > 17.1 g/L; albumin, < 35 or ≥ 35 g/L; ALT, < 2 times the upper normal limit or ≥ 2 times the upper normal limit; AST, < 2 times the upper normal limit or ≥ 2 times the upper normal limit; platelets, < 100 or ≥ 100 × 10⁹/L; prothrombin time, < 14 or ≥ 14 s; AFP, < 400 or ≥ 400

ng/mL; tumor size, < 5 or ≥ 5 cm; tumor capsule, present or absent; cirrhosis, present or absent; and Edmondson grade, I - II or III-IV.

Among all these variables, only the absence of tumor capsule showed a significant association with CD133 expression (*P* = 0.005; Table 3). CD90 expression was significantly more frequent in stage III or IV tumors than in stage I or II tumors (*P* = 0.010), and it was more frequent in larger tumors (*P* = 0.034). EpCAM expression was significantly more frequent in patients with elevated serum AFP levels (*P* = 0.021).

Association of CD133, CD90, and EpCAM expression with early recurrence

Univariate analysis showed that early HCC recurrence was associated with expression of CD90 (*P* = 0.001) and EpCAM (*P* = 0.045; Table 4). However, multivariate analysis showed that only CD90 expression correlated significantly with early recurrence (RR = 9.333; 95%CI: 2.207-39.463, *P* = 0.002).

Association of CD133, CD90, and EpCAM expression with overall survival

The association of CD133, CD90, and EpCAM expression with overall survival was evaluated by calculating Kaplan-Meier survival curves separately for patients positive or negative for each CSC marker and then comparing the curves using the log-rank test (Table 4). The survival curve analysis was then verified using Cox regression. Survival rates at one, two, and three years were similar between CD133-negative patients (87.5%, 72.9%, 54.7%) and CD133-positive patients (85.7%, 71.2%, 68.6%, *P* = 0.732; Figure 2A). In contrast, the corresponding survival rates were significantly higher for patients negative for CD90 expression (100%, 94.1%, 88.2%) than for CD90-positive patients (78.8%, 60.2%, 56.4%, *P* = 0.018; Figure 2B). Similarly, survival rates were significantly higher for EpCAM-negative patients (86.2%, 86.2%, and 82.3%) than for EpCAM-positive ones (85.7%, 51.3%, 46.2%, *P* = 0.010; Figure 2C). Multivariate Cox regression showed that only EpCAM expression (RR = 4.857; 95%CI: 1.648-14.313, *P* = 0.004) was a significant predictor of survival time in patients with HCC.

DISCUSSION

In this study, we evaluated the relationship between expression of three putative CSC markers and the most clinically relevant features of HCC. Our findings suggest that CD133, CD90, and EpCAM expression correlates with the onset and/or progression of HCC, because they are expressed to a significantly greater extent in HCC tissue than in normal liver tissue. In addition, EpCAM expression is associated with shorter survival, and CD90 expression predicts early recurrence.

The biology of several human cancers, including HCC, is driven by self-renewal, unlimited prolifera-

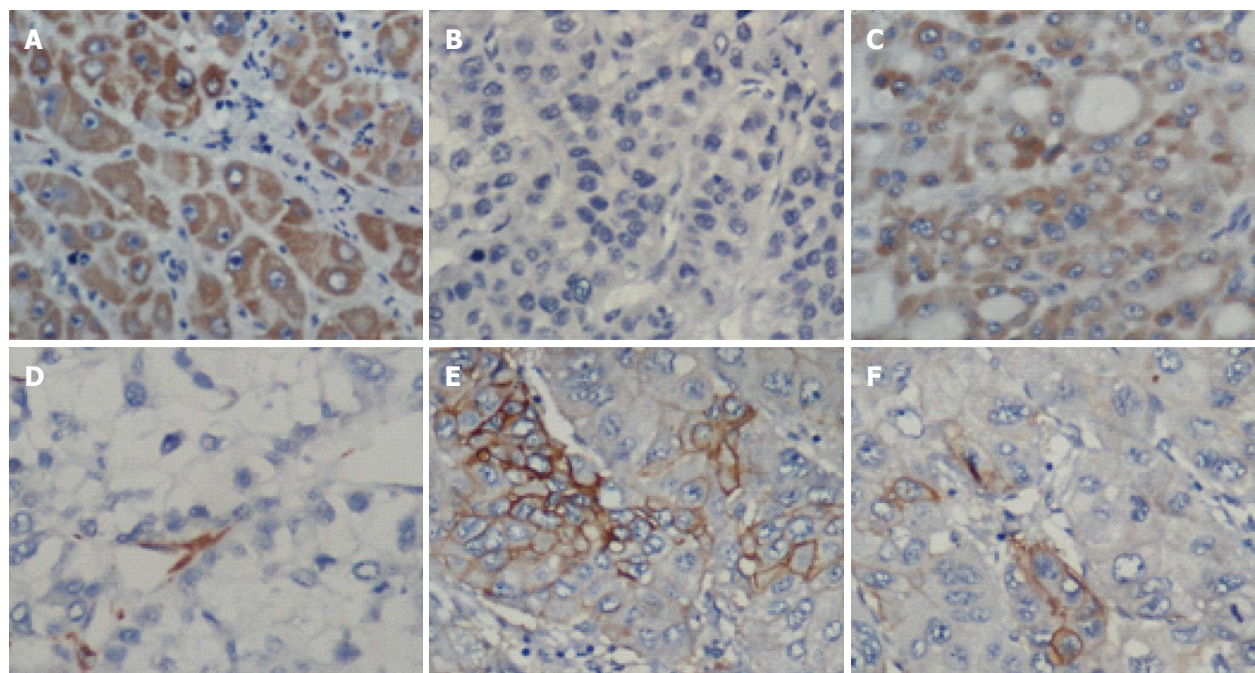


Figure 1 Representative images of hepatocellular carcinoma tissue. Representative images of hepatocellular carcinoma (HCC) tissue showing positive and negative cytoplasmic staining for CD133 (A, B) and CD90 (C, D), or positive and negative membrane staining for epithelial cell adhesion molecule (EpCAM) (E, F). Magnification, × 400.

Table 3 Association of CD133, CD90, and epithelial cell adhesion molecule expression with clinicopathological variables

Variable	n	CD133		P value	CD90		P value	EpCAM		P value
		Positive	Negative		Positive	Negative		Positive	Negative	
Tumor size (cm)										
< 5	22	20	2	0.439	11	11	0.034	11	11	0.310
≥ 5	28	22	6		22	6		10	18	
Tumor capsule										
Present	14	8	6	0.005	8	6	0.623	8	6	0.176
Absent	36	34	2		25	11		13	23	
Edmondson grade										
I or II	20	17	3	0.875	9	11	0.010	7	13	0.413
III or IV	30	25	5		24	6		14	16	
AFP (ng/mL)										
< 400	37	32	5	0.721	25	12	0.957	12	25	0.021
≥ 400	13	10	3		8	5		9	4	

Association of CD133, CD90, and epithelial cell adhesion molecule (EpCAM) expression with dichotomized clinicopathological variables in HCC patients after propensity-score matching; AFP: Alpha-fetoprotein.

tion, and differentiation, all of which are stem cell-like properties^[17]. In fact, CSCs have been proposed to initiate tumorigenesis and contribute to cancer resistance, metastasis, and recurrence^[18]. Several surface markers, including CD133, CD90, and EpCAM, have been identified as putative markers of LCSCs associated with HCC, though these markers are also present on other types of CSCs.

CD133, known as a 5-transmembrane domain glycoprotein, is expressed in various types of tumors^[19]. This marker has been used to identify and isolate CSCs in malignant cancers such as acute myeloid leukemia^[20] and brain and colon cancers^[21-23]. In addition, increased CD133 expression may be a prognostic marker in many human malignancies^[24-26]. Suetsugu *et al.*^[27] first identified

CD133 in HCC cells; those authors demonstrated that CD133-positive Huh-7 cells showed higher tumorigenic potential *in vivo* and greater proliferative ability *in vitro* than did CD133-negative cells. CD133 is now widely recognized as a CSC marker in HCC tissues. Our findings are consistent with this idea: in the 50 HCC samples that we examined, the average percentage of CD133-positive cells was 37.7% ± 26.0%. This percentage should be interpreted with caution, since not all CD133-positive cells correspond to LCSCs. Only a relatively small and well-defined subset of cells with enhanced ability to proliferate and form tumors should be considered CSCs^[9]. Identifying CSCs may require analyzing multiple markers. Indeed, a survey of HCC cell lines found that cells co-expressing both CD133 and CD44 were more likely

Table 4 Association of CD133, CD90, and epithelial cell adhesion molecule expression with recurrence and survival

Marker	ER group <i>n</i> = 25	NER group <i>n</i> = 25	<i>P</i> value	Overall survival			<i>P</i> value
				1 yr	2 yr	3 yr	
CD133							
Positive	20	22	0.440	85.9%	71.2%	68.6%	0.732
Negative	5	3		87.5%	72.9%	54.7%	
CD90							
Positive	22	11	0.001	78.8%	60.2%	56.4%	0.018
Negative	3	14		100.0%	94.1%	88.2%	
EpCAM							
Positive	14	7	0.045	85.7%	51.3%	46.2%	0.010
Negative	11	18		86.2%	86.2%	82.3%	

Association of CD133, CD90, and epithelial cell adhesion molecule (EpCAM) expression with dichotomized clinicopathological variables in HCC patients after propensity-score matching; ER: Early recurrence; NER: Non-ER.

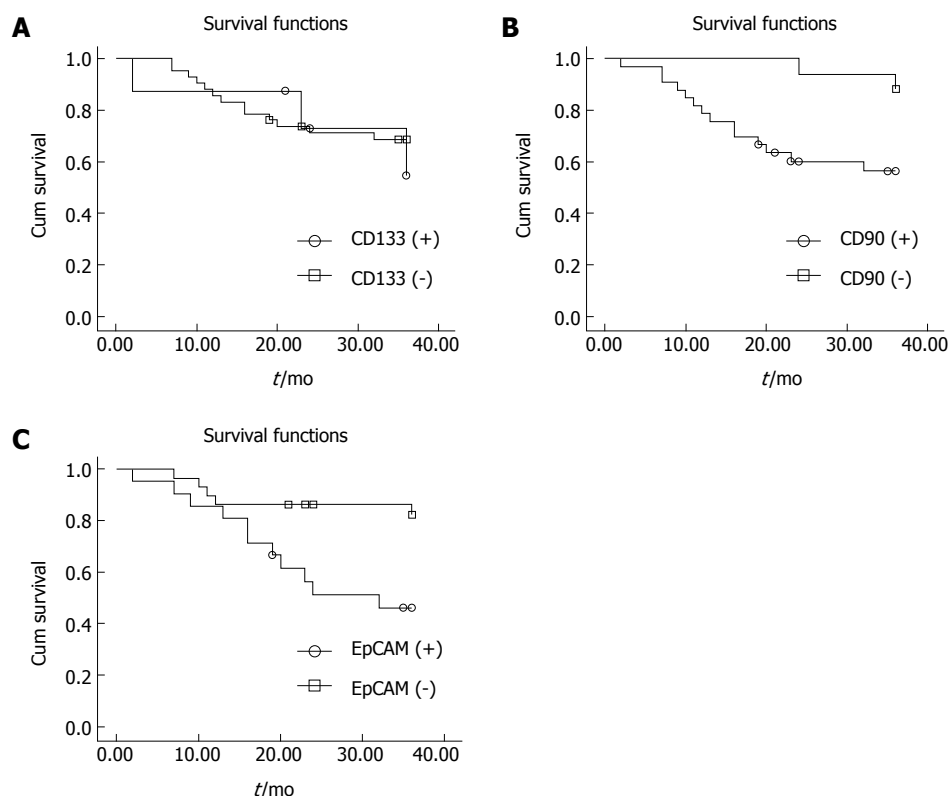


Figure 2 Overall survival curves. A: For patients whose hepatocellular carcinoma (HCC) tissue was positive or negative for CD133 expression. The two curves were not significantly different, based on the log-rank test ($P = 0.732$); B: For patients whose HCC tissue was positive or negative for CD90 expression. Survival times were significantly shorter for CD90-positive patients ($P = 0.018$); C: For patients whose HCC tissue was positive or negative for epithelial cell adhesion molecule (EpCAM) expression. Survival times were significantly shorter for EpCAM-positive patients ($P = 0.010$).

to be LCSCs than cells expressing CD133 alone^[28].

In our study, CD133 expression was associated with the absence of tumor capsule. The tumor capsule acts as a barrier to prevent the spread of tumor cells^[29], giving it an important role in tumor suppression. Our findings suggest that CD133 tends to be expressed in tumors showing stronger potential for invasion and metastasis.

CD90, a cell surface glycoprotein of 25-37 kDa, plays an important role in cell-cell and cell-matrix interactions^[30]. The protein has been used as a surface marker of many types of stem cells^[31,32]. CD90-positive cells isolated from HCC cell lines, human HCC specimens, and blood samples are tumorigenic in a mouse xenograft

model, suggesting that CD90 is also an LCSC marker^[33]. Here we report that CD90 expression is not only significantly higher in HCC tissues than in normal adult liver tissue, but also correlates with higher histopathologic grade and larger tumors. These findings are similar to a former study^[34] which found higher expression of CD90 in poorly differentiated HCC than in well-differentiated ones with staining intensity correlating to degree of differentiation. These results suggest that CD90 is involved in the onset and/or progression of HCC.

EpCAM is another cell surface glycoprotein, and it functions as a homophilic, epithelial-specific intercellular adhesion molecule^[35,36]. More recent work has shown

that the protein also contributes to cell signaling, proliferation, differentiation, and migration^[37,38]. Yamashita *et al.*^[39] were the first to characterize EpCAM in HCC cell lines and tumor specimens; they demonstrated that EpCAM-positive HCC cells possess LCSC-like abilities to self-renew and differentiate. In the present study, we show that EpCAM expression is significantly higher in HCC tissues than in normal adult liver tissues, and that it correlates with elevated serum AFP levels. Since AFP level correlates with the degree of HCC malignancy^[40], these findings indicate that EpCAM tends to be expressed in more malignant HCC tissues. Therefore EpCAM may well play a role in HCC progression.

Postoperative recurrence is the main cause of death for HCC patients in the long term^[7,8], and most recurrences occur within two years after resection^[16]. Evidence suggests that CSCs may drive postoperative recurrence and metastasis^[41]. First, CSCs detach from the primary mass and enter the lymph and peripheral blood. Then they sense a chemoattractant gradient that directs them to a particular point, where they attach to the endothelium and penetrate the microvessel wall. Outside the vasculature, CSCs find an environmental niche that protects them from damage and allows them to establish a recurrent or metastatic tumor.

Based on the literature, we defined two years as a cut-off for early recurrence, and we divided our patients into two groups: those who experienced early recurrence ($n = 38$) and those who did not ($n = 52$). Since the two groups showed several significant differences at baseline (Table 1), as has been observed to be related to tumor recurrence in other studies of HCC^[13,16], we generated balanced pairs of ER and NER patients using propensity-score matching. Analysis of these 25 pairs showed that CD90 and EpCAM were expressed to a significantly greater degree in ER patients than in NER patients; CD133 expression, however, was similar between the two groups. Multivariate analysis showed that of the three putative LCSC markers, only expression of CD90 was significantly associated with early recurrence. This finding is consistent with previous work linking CD90 up-regulation to HCC tumor invasion and metastasis^[34]. In that study, CD90-positive cells were found to be more likely than CD90-negative cells to detach from the primary tumor and establish recurrent tumors in appropriate environmental niches in the residual liver.

In the present study, we also evaluated the ability of CD133, CD90, and EpCAM to predict survival time in HCC patients. Univariate analysis showed expression of both CD90 and EpCAM to be associated with shorter survival time, while multivariate analysis showed only EpCAM expression to predict shorter survival time. Expression of EpCAM has also been associated with shorter survival time in cancers of the breast^[42], renal clear cells^[43], ovaries^[44], and gallbladder^[45]. The poor prognosis associated with EpCAM expression may indicate that EpCAM-positive cells possess the CSC properties of self-renewal, unlimited proliferation and differentiation.

The present study has several limitations, including small cohort size from a single site, short follow-up, and an observational design. Future studies should verify the insights from the present work and extend them by defining the optimal mixture of surface markers (including CD133) for identifying and isolating LCSCs. Future studies should also explore how CD133, CD90, and EpCAM - and potentially other CSC markers - influence postoperative recurrence and prognosis in patients with HCC.

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COMMENTS

Background

The cancer stem cell (CSC) hypothesis stipulates that primary tumors are initiated and maintained by a small population of cancer cells with "stem cell-like" characteristics. And it also states that tumor recurrence and metastasis after surgical resection are driven by CSCs. Recently, numerous cell surface markers, such as CD133, CD90, and epithelial cell adhesion molecule (EpCAM), have been identified as CSC markers in hepatocellular carcinoma (HCC). However, the association of CSC markers with early HCC recurrence and survival time is still unclear.

Research frontiers

Liver cancer stem cells (LCSCs) have been identified by numerous surface markers, including CD133, CD90 and EpCAM. However, definite LCSC markers are still controversial, because none of these markers are exclusively expressed by LCSCs in HCC. In addition, the research hotspot is to develop specific therapies targeting LCSCs.

Innovations and breakthroughs

To date, many clinical researches have attempted to investigate whether the existence of LCSCs is associated with clinical outcomes in HCC. However, the clinical relevance of LCSCs remains a major challenge for current anti-cancer therapy. In this study, authors aimed to analyze the expression of CD133, CD90 and EpCAM in patients and to search for association with early recurrence and survival time. To reduce the bias in patient selection, propensity-score matching was used to generate pairs of early recurrence (ER) and non-ER patients. The data indicated that EpCAM and CD90 are associated with shorter survival time and early HCC recurrence, respectively.

Applications

The study results suggested that the expression of the three LCSC markers CD133, CD90 and EpCAM is linked to HCC tumor onset and/or progression. In addition, EpCAM is associated with shorter survival time, while CD90 is associated with early recurrence.

Peer review

This is an interesting study in which the authors investigated the association of three CSC markers CD133, CD90 and EpCAM with the early HCC recurrence and survival time in patients with HCC. The results suggested that the EpCAM expression is associated with shorter survival time, while the expression of CD90 is associated with early recurrence.

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