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Original Article

The aberrant expressions of MACC1, ZEB1, and KLF4 in hepatocellular carcinoma and their clinical significance

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Abstract: Background and objective: Metastasis-associated in colon cancer-1 (MACC1) is involved in the progression and metastasis of various cancers. Zinc finger E-box-binding homeobox 1 (ZEB1) is a key transcriptional factor of the epithelial-mesenchymal transition (EMT) that is involved in the migration and invasion of cancer cells. Kruppel-like factor 4 (KLF4) is a tumor suppressor that can inhibit tumor cell proliferation, migration, and metastasis. The purpose of this study was to investigate the expressions and clinical significance of MACC1, ZEB1, and KLF4 in hepatocellular carcinoma (HCC). Methods: We analyzed the expressions of MACC1, ZEB1, and KLF4 in 153 HCC specimens and their corresponding control specimens. The patients' clinicopathological and follow-up data were also collected. Results: The rates of positive expression of MACC1 and ZEB1 were significantly higher in the HCC specimens than in the control specimens, and their expressions were positively associated with the number of tumors, grades of differentiation, lymph node metastasis (LNM), and tumor-node-metastasis (TNM) stages. Inversely, the rate of positive expression of KLF4 was significantly lower in the HCC specimens than it was in the control specimens, and its expression was negatively correlated with the number of tumors, grades of differentiation, LNM, and TNM stages. The patients who expressed MACC1 or ZEB1 had a reduced overall survival (OS) when compared with patients not expressing these proteins. However, the patients who expressed KLF4 had an increased OS when compared with patients who did not show any KLF4 expression. A multivariate analysis indicated that the expressions of MACC1, ZEB1, and KLF4 and tumor size, LNM, as well as the TNM stages were independent, prognostic factors for HCC patients. Conclusions: Therefore, positive expressions of MACC1, ZEB1, and KLF4 should be correlated with the duration of OS in patients with HCC and considered promising prognostic biomarkers, as well as potential therapeutic targets for HCC.

Keywords: HCC, MACC1, ZEB1, KLF4, prognosis

Introduction

New liver cancer cases were estimated at 783,000, and deaths were estimated at 746,000 worldwide in 2012 [1]. But in China, new liver cancer cases were estimated at 466,000 and deaths were estimated at 422,000 in 2015 [2], accounting for approximately 50% of the total number of new cases and deaths. The most common type of liver cancer is hepatocellular carcinoma (HCC). Many HCC patients are diagnosed at the advance stages in China because the disease does not show any apparent symptoms during the early stages.

Relapse and metastasis are the most common reasons for liver cancer treatment failure. Metastasis-associated in colon cancer 1 (MACC1) is considered an oncogene that was originally identified in the colon cancer cell line in 2009 [3]. Previous studies have demonstrated that MACC1 is able to induce the epithelial-mesenchymal transition (EMT) to promote tumor cell invasion and metastasis both in vitro and in vivo [3-6]. MACC1 is a key regulator of the hepatocyte growth factor (HGF)/MET signaling pathway that can bind to the promoter of the mesenchymal-epithelial transition (MET) gene to regulate its transcriptional activity [3, 7]. It was reported that MACC1 is an effective and valuable biomarker in various types of cancer, especially in the prediction of metastasis and prognosis [8].

It is well known that EMT is one of the key mechanisms of carcinoma metastasis [9]. EMT occurs when epithelial cancer cells lose their

Table 1. The patients' characteristics

Patients characteristics	Frequency (n)	Percentage (%)
Age (years)		
< 60	68	44.4
≥ 60	85	55.6
Gender		
Male	117	76.5
Female	36	23.5
Alcohol		
No	91	59.5
Yes	62	40.5
Size (cm)		
< 2.0	47	30.7
≥ 2.0, < 5.0	71	46.4
≥ 5.0	35	22.9
HBSAg		
No	86	56.2
Yes	67	43.8
Number of tumors		
1	82	53.6
> 1	71	46.4
Cirrhosis		
No	86	56.2
Yes	67	43.8
Grades		
Well + moderate	77	50.3
Poor	76	49.7
Lymph node metastasis		
N0	147	96.1
Yes	6	3.9
TNM stages		
I	52	34.0
II	64	41.8
III	31	20.3
IV A	6	3.9

epithelial features and acquire mesenchymal features that can promote invasion and metastasis [10, 11]. Zinc finger E-box binding homeobox 1 (ZEB1), which is a key transcriptional regulator of EMT, consists of two zinc finger clusters and a centrally-located homeodomain responsible for DNA binding [12]. ZEB1 is able to induce tumor cell invasion and metastasis by promoting EMT [13]. The overexpression of ZEB1 is often found in various cancers and may be considered a metastatic and prognostic biomarker for many cancers [13-15].

Kruppel-like factor 4 (KLF4) is a zinc finger transcriptional factor that was originally identified as being expressed in the epithelial cells of the skin and intestines [16]. KLF4 can bind to the GC or CACCC-rich DNA sequences to regulate cell proliferation, differentiation, and apoptosis [17]. However, KLF4 plays dual functions in tumorigenesis and development since it can serve as a tumor suppressor or an oncogene [18, 19]. For example, KLF4 has been shown to function as an oncogene in many tumors, such as skin squamous cell carcinoma and breast carcinoma [20, 21]. A suppressed role of KLF4 was found in HCC, lung cancer, and gastric cancer [16, 22, 23].

Although these biomarkers are widely recognized in tumor initiation, progression and metastasis, studies on the role of these biomarkers in HCC remain unclear. The purpose of this study is to evaluate the hypothesis that these biomarkers associate with HCC progression and prognosis.

Methods

Patients and specimens

We recruited 153 recorded patients who were diagnosed with HCC from January 2012 to December 2013 by the Department of Pathology of our hospital and collected samples of cancer tissue and the corresponding normal liver tissue from all patients. This study is retrospective. All patients who had any history of chemo- or radio-therapy or other anti-cancer therapy were excluded. All HCC patients provided written informed consents. This study was authorized by the ethics committee of Bengbu Medical University and performed in accordance with the Declaration of Helsinki before it started. The patient data included clinicopathologic, demographic, and follow-up data (follow-up by phone or social applications). The follow-up data was calculated from the date of surgery to his/her death date or to December 2017. The TNM stages were evaluated in accordance with the 8th edition of guidelines issued by the AJCC (American Joint Committee on Cancer). Grades of differentiation were evaluated in accordance with the guidelines issued by the WHO (World Health Organization). The specific characteristics are shown in **Table 1**.

Immunohistochemistry

All the HCC tissues and normal liver tissues were fixed in 10% buffered formalin solution

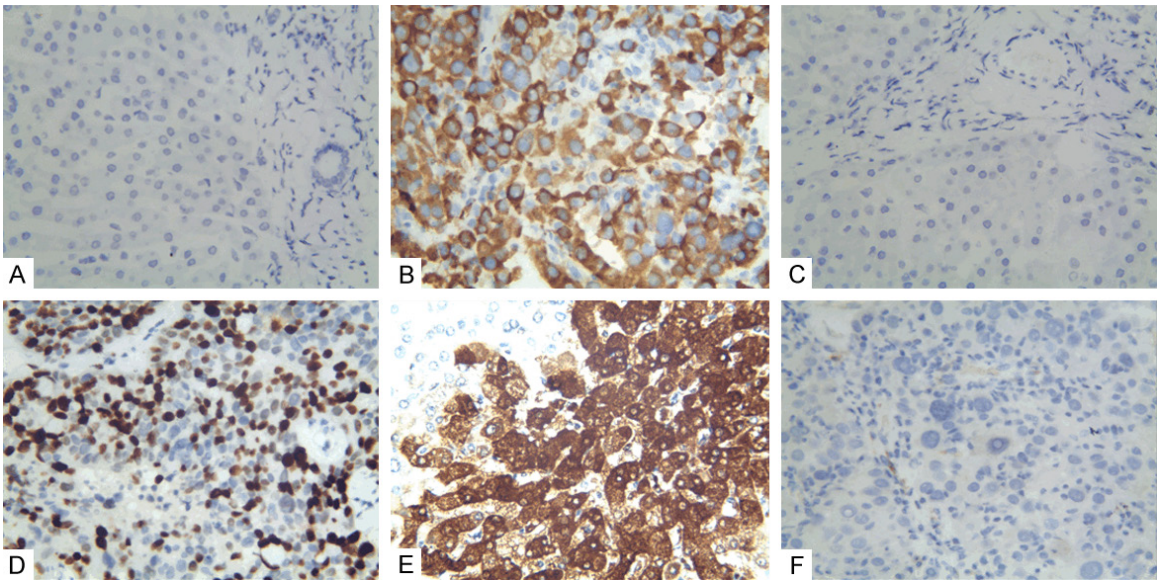

Figure 1. Immunostaining of MACC1, ZEB1, and KLF4 in HCC and the control tissue (400 magnification). A. Negative staining MACC1 in the control tissue; B. Positive staining of MACC1 in the cytoplasms of cancer cells; C. Negative staining of ZEB1 in the control tissue; D. Positive staining of ZEB1 in the nuclei of cancer cells; E. Positive staining of KLF4 in the cytoplasms and nuclei of the control cells; F. Negative staining of KLF4 in the cancer cells.

and then embedded in paraffin. Continuous 4-μm-thick sections were cut. Immunohistochemical staining was performed using the Elivision™ Plus method. The immunostaining procedure was performed following the kit's instructions. Endogenous peroxidase activity was blocked by methanol containing a 3% H_2O_2 solution. The antigen repair used a citrate buffer solution (pH 6.0). Then all the sections were blocked with goat serum. Rabbit polyclonal antibody against human MACC1 (Santa Cruz, CA, USA) and ZEB1 (Abcam, MA, USA) and mouse monoclonal antibody against human KLF4 (Abcam, MA, USA) primary antibodies were added, then all sections were incubated at 4°C overnight. All the sections were developed in a diaminobenzidine (DAB) substrate solution. Finally, all the slices were re-dyed with hematoxylin and mounted with gum.

Evaluation of staining

Ten high-power-fields (HPF) were randomly selected to avoid intratumoral heterogeneity of biomarker expression. The immunostaining was interpreted by two pathologists who were blinded to the patients' data and evaluated by semi-quantitative points. The staining results were scored in accordance with the staining intensity and staining extent. The staining intensity scoring was done as follows: no staining was 1; weak staining was 2; moderate staining was 2; and strong staining was 3. The staining extent scoring was done as follows: < 11% positive cells was 1; 11-50% positive cells was 2; 51-75% positive cells was 3; and > 75% positive cells was 4. The final scores (range 0-12) were calculated by multiplying the intensity score by the extent score. The expression was considered positive when the score was > 2. For tissues that were positive for MACC1, ZEB1 and KLF4, an average of the final score of each was taken.

Statistical analysis

All data were analyzed using SPSS 19.0 software (Chicago, IL, US). The countable data were subjected to a Chi-square test or Fisher's exact test for comparisons between two groups. Univariate OS time analysis was performed using the Kaplan-Meier method with a log-rank test. Multivariate OS time analysis was performed using a Cox regression model test. $P < 0.05$ was defined as being indicative of statistically significant differences.

Results

The expressions of MACC1, ZEB1, and KLF4 in HCC, and their relationships to clinicopathologic characteristics

As shown in **Figure 1A** and **1B**, the MACC+ expression was mainly situated in the cytoplasms. The MACC1+ expression in the HCC cells (60.8%, 93/153) was significantly higher

Table 2. The associations between the expressions of MACC1, ZEB1, and KLF4 and the clinicopathological characteristics of hepatocellular carcinoma (HCC)

Variables	MACC1		P	ZEB1		P	KLF4		P
	-	+		-	+		-	+	
Age (years)			0.120			0.141			0.856
< 60	22	47		32	36		35	33	
≥ 60	38	46		30	55		45	40	
Gender			0.224			0.075			0.753
Male	49	68		52	65		62	55	
Female	11	25		10	26		18	18	
Alcohol			0.916			0.706			0.848
No	36	55		38	53		47	44	
Yes	24	38		24	38		33	29	
Size (cm)			0.246			0.206			0.160
< 2.0	22	25		24	23		30	17	
≥ 2.0, < 5.0	28	43		25	46		34	37	
≥ 5.0	10	25		13	22		16	19	
HBSAg			0.671			0.168			0.196
No	35	51		39	47		41	45	
Yes	25	42		23	44		39	28	
Number			< 0.001			< 0.001			< 0.001
1	54	28		58	24		30	52	
> 1	6	65		4	67		50	21	
Cirrhosis			0.078			0.703			0.196
No	39	47		36	50		41	45	
Yes	21	46		26	41		39	28	
Grades			< 0.001			< 0.001			< 0.001
Well + moderate	44	33		44	33		29	48	
Poor	16	60		18	58		51	25	
LNM			0.082			0.082			0.029
No	60	87		62	85		74	73	
Yes	0	6		0	6		6	0	
TNM stages			< 0.001			< 0.001			< 0.001
I	44	8		44	8		14	38	
II	7	57		7	57		46	18	
III	9	22		22	20		14	17	
IV A	0	6		0	6		6	0	

than it was in the control cells (6.5%, 10/153; P < 0.001). The MACC1 expression was positively related to the number of tumors, grades of differentiation, and TNM stages. In contrast, there were no relationships between MACC1+ expression and patient age, gender, tumor size, alcohol status, HBSAg status, lymph node metastasis (LNM), or cirrhosis (P > 0.05; **Table 2**).

As shown in **Figure 1C** and **1D**, ZEB1+ expression was mainly situated at the nuclei. Similar to MACC1, the ZEB1+ expression in the HCC cells (59.5%, 91/153) was significantly higher than it was in the control cells (3.3%, 5/153; P < 0.001). Furthermore, the ZEB1+ expression positively related to the number of tumors, grades of differentiation, and TNM stages. And at the same time, there were no relationships between ZEB1+ expression and patient age, gender, tumor size, alcohol status, HBSAg status, LNM, or cirrhosis (P > 0.05; **Table 2**).

As shown in **Figure 1E** and **1F**, KLF4+ expression was mainly situated in the nuclei and cytoplasms. Inversely to MACC1, the KLF4+ expression in HCC cells (47.7%, 73/153) was significantly lower than it was in the control cells (81.0%, 124/153; P < 0.01). Moreover, the KLF4+ expression was positively related to the number of tumors, grades of differentiation, LNM, and TNM stages. And there were no relationships between KLF4+ expression and patient age, gender, tumor size, alcohol status, HBSAg status, or cirrhosis (P > 0.05; **Table 2**).

The relationships among MACC1, ZEB1, and KLF4 in HCC

The spearman correlation coefficient analysis indicated a negative association between KLF4+ expression, and the MACC1+ or ZEB1+ expression was negative (r = -0.466; r = -0.304; respectively; both P < 0.001). There was a positive association between MACC1+ expression and ZEB1+ expression (r = 0.482, P < 0.001) (**Table 3**).

Table 3. The correlation among the expressions of MACC1, ZEB1, and KLF4 in HCC

Variable	MACC1		r	P	ZEB1		r	P
	-	+			-	+		
MACC1							0.482	< 0.001*
-					42	18		
+					20	73		
KLF4			-0.466	< 0.001@			-0.304	< 0.001@
-	14	66			21	59		
+	46	27			41	32		

*: positive association; @: negative association.

Survival analysis

As shown in **Figure 2A**, a Kaplan-Meier analysis demonstrated that the OS time of the HCC patients who expressed MACC1 (26.7 ± 13.6 months) was significantly lower than that of the patients who did not express the protein (50.1 ± 14.7 months; log-rank = 56.588, P < 0.001). As shown in **Figure 2B**, the OS time for the ZEB1-positive patients (29.0 ± 14.7 months) was significantly lower than the OS time of the ZEB1-negative patients (46.0 ± 17.9 months; log-rank = 38.518, P < 0.001). As shown in **Figure 2C**, the OS time of the patients who expressed KLF4 (47.0 ± 14.3 months) was significantly higher than the OS time of the patients did not express protein (25.7 ± 14.9 months; log-rank = 43.181, P < 0.001). As shown in **Figure 2D**, the OS time of the patients who expressed a combination of KLF4-negative, MACC1-positive, and ZEB1-positive was significantly lower than the OS time of the patients who expressed KLF4-positive, MACC1-negative, and ZEB1-negative (log-rank = 111.165, P < 0.001).

A multivariate analysis demonstrated that the expressions of MACC1, ZEB1, and KLF4 and tumor size, LNM, as well as TNM stages were independent prognostic factors for HCC (**Table 4**).

Discussion

HCC is a common cancer of the digestive system and is a highly heterogeneous cancer. The heterogeneity of HCC makes it difficult to evaluate the effectiveness of biomarkers. It has been demonstrated that MACC1 not only stimulates cell proliferation, mobility, migration, and metastasis in vitro, but it also promotes cell growth, migration, and metastasis in vivo [3, 6, 7, 24]. In this study, we detected MACC1 expression in HCC and the corresponding normal liver tissues and found that the HCC tissue expressed higher levels of the protein than the control tissues. Furthermore, MACC1 expression positively related to the number of tumors, grades of differentiation, and TNM stages. The OS analysis showed that HCC patients expressing MACC1 survived for less time than patients who did not express the protein. The above results suggest that MACC1 expression in this study should be similar to previous studies in HCC [25, 26], and they also suggest that MACC1 should be considered a usefully prognostic biomarker for HCC.

ZEB1, which consists of two zinc finger clusters and a centrally-located homeodomain, is a critical transcriptional regulator of EMT [12, 13]. Previous studies have demonstrated that the overexpression of ZEB1 should promote tumor cell migration, invasion, metastasis, as well as the EMT [26, 27]. In this study, ZEB1 overexpression was significantly related to the number of tumors, grades of differentiation, and TNM stages. Furthermore, the OS analysis indicated that the patients who expressed ZEB1 survived for less time than those who did not express ZEB1. These results are similar to other studies suggesting that ZEB1 should be a usefully prognostic biomarker for HCC [13, 28, 29].

It has been demonstrated that KLF4 plays a dual function as either a tumor suppressor or an oncogene in tumorigenesis [18, 30]. In this study, our results demonstrated that KLF4 expression was inversely related to the number of tumors, grades of differentiation, LNM, as well as the TNM stages. In addition, the OS analysis indicated that patients expressing KLF4 lived longer than those who did not express KLF4. Our results are similar to the previous studies in HCC [22, 31, 32] and suggest that KLF4 should be considered a useful and valuable biomarker for the prediction of progression and prognosis.

In this study, a Kaplan-Meier analysis showed that patients who expressed MACC1, ZEB1, KLF4, or the co-expressions of MACC1, ZEB1, and KLF4 survived less or longer than those

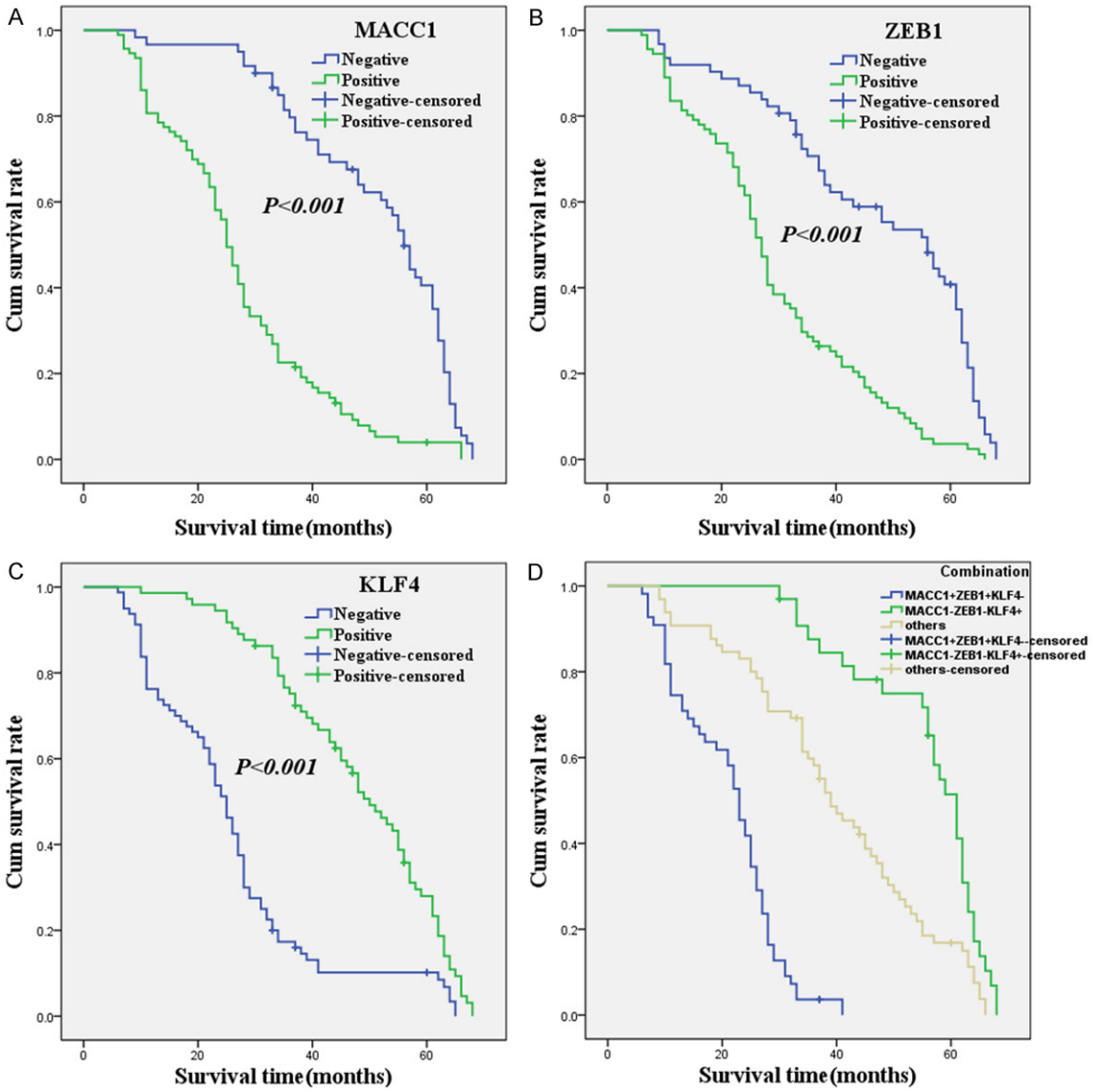

Figure 2. A Kaplan-Meier analysis of the survival rate of patients with HCC. (A) Overall survival of all patients in relation to MACC1 expression (log-rank = 56.588, P < 0.001); (B) Overall survival of all patients in relation to ZEB1 (log-rank = 38.518, P < 0.001); (C) Overall survival of all patients in relation to KLF4 (log-rank = 43.181, P < 0.001); In (A-C) analyses, the green line represents the positive staining of factors and the blue line represents the negative staining factors. (D) Overall survival of all patients in relation to the combination of KLF4, MACC1, and ZEB1 (log-rank = 111.165, P < 0.001). The green line represents the positive expression of KLF4 and the negative expression of MACC1 and ZEB1 and the blue line represents the negative expression of KLF4 and the positive expression of MACC1 and ZEB1. The red line represents other positive or negative expressions of the proteins.

Table 4. Results of the multivariate analyses of overall survival (OS) time

Covariate	B	SE	P	HR	95% CI
MACC1	0.500	0.247	0.043	1.648	1.016-2.675
ZEB1	0.594	0.274	0.030	1.812	1.060-3.097
KLF4	-1.705	0.229	< 0.001	0.182	0.116-0.285
Tumor size	0.631	0.186	0.001	1.879	1.305-2.706
LNM	1.163	0.562	0.039	3.199	1.063-9.628
TNM stages	1.054	0.210	< 0.001	2.869	1.903-4.326

who did not express these proteins. A multivariate analysis showed that the expressions of MACC1, ZEB1, and KLF4, and tumor size, LNM, as well as TNM stages were independent prognostic factors for HCC patients. Our findings also suggested that the expressions of MACC1, ZEB1, and KLF4 should be considered useful and valuable biomarkers for predicting the progression and prog-

nosis of HCC. A normal expression of KLF4 can inhibit the expression of the EMT-related proteins ZEB1, snail, and slug by activating the expressions of miR-153, miR-506, and miR-200b [33]. Down- or lost-regulation of KLF4 should be involved in the initiation of tumorigenesis and increase cell proliferation, invasion, and metastasis [17]. Meanwhile, the overexpression of MACC1 should be involved in tumorigenesis by promoting cell proliferation and also promote tumor cell invasion and metastasis by the activation of EMT via the HGF/MET signaling pathways [8, 34]. ZEB1 can act as a driver of EMT through the inhibition of E-cadherin expression and the activation of the Fak/Src signal pathway [35, 36]. Thus, EMT and the aberrant expression of KLF4 should promote cancer cell mobility, migration, and metastasis. The OS time of patients who expressed the combination of being KLF4-negative, MACC1-positive, and ZEB1-positive was significantly lower than the OS time of the patients who expressed KLF4-positive, MACC1-negative, and ZEB1-negative. This result suggests that the combined detection of KLF4, MACC1, and ZEB1 expression in HCC may predict patients' prognosis at the early stages.

Conclusions

This study found that positive expressions of MACC1, ZEB1, and KLF4 are associated with time of OS among patients with HCC. Therefore, MACC1, ZEB1, and KLF4 should be thought of as useful and valuable biomarkers to predict metastasis and prognosis in HCC patients.

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Disclosure of conflict of interest

None.

Abbreviations

MACC1, metastasis-associated in colon cancer 1; HCC, hepatocellular carcinoma; ZEB1, Zinc finger E-box-binding homeobox 1; KLF4, Kruppel-like factor 4; EMT, epithelial-mesenchymal transition; HGF/MET, hepatocyte growth factor/mesenchymal-epithelial transition; LNM, lymph node metastasis; TNM, tumor-node-metastasis; OS, overall survival; HPF, high-power-field.

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References

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

[2] Chen W, Zheng R, Baade PD, Zhang S, Zeng H, Bray F, Jemal A, Yu XQ, He J. Cancer statistics in China, 2015. CA Cancer J Clin 2016; 66: 115-32.

[3] Stein U, Walther W, Arlt F, Schwabe H, Smith J, Fichtner I, Birchmeier W, Schlag PM. MACC1, a newly identified key regulator of HGF-MET signaling, predicts colon cancer metastasis. Nat Med 2009; 15: 59-67.

[4] Koelzer VH, Herrmann P, Zlobec I, Karamitopoulou E, Luqli A, Stein U. Heterogeneity analysis of metastasis associated in colon cancer 1 (MACC1) for survival prognosis of colorectal cancer patients: a retrospective cohort study. BMC Cancer 2015; 15: 160.

[5] Wang L, Wu Y, Lin L, Liu P, Huang H, Liao W, Zheng D, Zuo Q, Sun L, Huang N, Shi M, Liao Y, Liao W. Metastasis-associated in colon cancer-1 upregulation predicts a poor prognosis of gastric cancer, and promotes tumor cell proliferation and invasion. Int J Cancer 2013; 133: 1419-30.

[6] Chundong G, Uramoto H, Onitsuka T, Shimokawa H, Iwanami T, Nakagawa M, Oyama T, Tanaka F. Molecular diagnosis of MACC1 status in lung adenocarcinoma by immunohistochemical analysis. Anticancer Res 2011; 31: 1141-5.

[7] Stein U, Smith J, Walther W, Arlt F. MACC1 controls Met: what a difference an Sp1 site makes. Cell Cycle 2009; 8: 2467-9.

[8] Zhu B, Wang Y, Wang X, Wu S, Zhou L, Gong X, Song W, Wang D. Evaluation of the correlation of MACC1, CD44, Twist1, and KiSS-1 in the metastasis and prognosis for colon carcinoma. Diagn Pathol 2018; 13: 45.

[9] Zhu Y, Zhang W, Wang P. Smoking and gender modify the effect of TWIST on patient survival in head and neck squamous carcinoma. Oncotarget 2017; 8: 85816-27.

[10] Yang D, Sun Y, Hu L, Zheng H, Ji P, Pecot CV, Zhao Y, Reynolds S, Cheng H, Rupaimoole R, Cogdell D, Nykter M, Broaddus R, Rodriguez-

Aguayo C, Lopez-Berestein G, Liu J, Shmulevich I, Sood AK, Chen K, Zhang W. Integrated analyses identify a master microRNA regulatory network for the mesenchymal subtype in serous ovarian cancer. Cancer Cell 2013; 23: 186-99.

[11] Mitra R, Chen X, Greenawalt EJ, Maulik U, Jiang W, Zhao Z, Eischen CM. Decoding critical long non-coding RNA in ovarian cancer epithelial-to-mesenchymal transition. Nat Commun 2017; 8: 1604.

[12] Onder TT, Gupta PB, Mani SA, Yang J, Lander ES and Weinberg RA. Loss of E-cadherin promotes metastasis via multiple downstream transcriptional pathways. Cancer Res 2008; 68: 3645-54.

[13] Han L, Chen W, Xia Y, Song Y, Zhao Z, Cheng H, Jiang T. MiR-101 inhibits the proliferation and metastasis of lung cancer by targeting zinc finger E-box binding homeobox 1. Am J Transl Res 2018; 10: 1172-83.

[14] Zhou YM, Cao L, Li B, Zhang RX, Sui CJ, Yin ZF, Yang JM. Clinicopathological significance of ZEB1 protein in patients with hepatocellular carcinoma. Ann Surg Oncol 2012; 19: 1700-1706.

[15] Zhang J, Lu C, Zhang J, Kang J, Cao C and Li M. Involvement of ZEB1 and E-cadherin in the invasion of lung squamous cell carcinoma. Mol Biol Rep 2013; 40: 949-956.

[16] Jia Y, Ying X, Zhou J, Chen Y, Luo X, Xie S, Wang QC, Hu W, Wang L. The novel KLF4/PLAC8 signaling pathway regulates lung cancer growth. Cell Death Dis 2018; 9: 603.

[17] Kaczynski J, Cook T, Urrutia R. Sp1- and Krüppel-like transcription factors. Genome Biol 2003; 4: 206.

[18] Tetreault MP, Yang Y, Katz JP. Krüppel-like factors in cancer. Nat Rev Cancer 2013; 13: 701-13.

[19] Ghaleb AM, Yang VW. Krüppel-like factor 4 (KLF4): what we currently know. Gene 2017; 611: 27-37.

[20] Huang CC, Liu Z, Li X, Bailey SK, Nail CD, Foster KW, Frost AR, Ruppert JM, Lobo-Ruppert SM. KLF4 and PCNA identify stages of tumor initiation in a conditional model of cutaneous squamous epithelial neoplasia. Cancer Biol Ther 2005; 4: 1401-8.

[21] Foster KW, Frost AR, McKie-Bell P, Lin CY, Engler JA, Grizzle WE, Ruppert JM. Increase of GKLF messenger RNA and protein expression during progression of breast cancer. Cancer Res 2000; 60: 6488-95.

[22] Yang X, Zhang D, Liu S, Li X, Hu W, Han C. KLF4 suppresses the migration of hepatocellular carcinoma by transcriptionally upregulating monoglyceride lipase. Am J Cancer Res 2018; 8: 1019-29.

[23] Hsu LS, Chan CP, Chen CJ, Lin SH, Lai MT, Hsu JD, Yeh KT, Soon MS. Decreased Kruppel-like factor 4 (KLF4) expression may correlate with poor survival in gastric adenocarcinoma. Med Oncol 2013; 30: 632.

[24] Ren B, Zakharov V, Yang Q, McMahon L, Yu J, Cao W. MACC1 is related to colorectal cancer initiation and early-stage invasive growth. Am J Clin Pathol 2013; 140: 707-7.

[25] Feng J, Wang J, Chen M, Chen G, Wu Z, Ying L, Zhuo Q, Zhang J, Wang W. miR-200a suppresses cell growth and migration by targeting MACC1 and predicts prognosis in hepatocellular carcinoma. Oncol Rep 2015; 33: 713-20.

[26] Wang H, Huang B, Li BM, Cao KY, Mo CQ, Jiang SJ, Pan JC, Wang ZR, Lin HY, Wang DH, Qiu SP. ZEB1-mediated vasculogenic mimicry formation associates with epithelial-mesenchymal transition and cancer stem cell phenotypes in prostate cancer. J Cell Mol Med 2018; 22: 3768-81.

[27] Liu W, Huang YJ, Liu C, Yang YY, Liu H, Cui JG, Cheng Y, Gao F, Cai JM, Li BL. Inhibition of TBK1 attenuates radiation-induced epithelial-mesenchymal transition of A549 human lung cancer cells via activation of GSK-3β and repression of ZEB1. Lab Inves 2014; 94: 362-70.

[28] Zhou YM, Cao L, Li B, Zhang RX, Sui CJ, Yin ZF, Yang JM. Clinicopathological significance of ZEB1 protein in patients with hepatocellular carcinoma. Ann Surg Oncol 2012; 19: 1700-06.

[29] Shen A, Zhang Y, Yang H, Xu R, Huang G. Overexpression of ZEB1 relates to metastasis and invasion in osteosarcoma. J Surg Oncol 2012; 105: 830-4.

[30] Rowland BD, Peeper DS. KLF4, p21 and context-dependent opposing forces in cancer. Nat Rev Cancer 2006; 6: 11-23.

[31] Sun H, Peng Z, Tang H, Xie D, Jia Z, Zhong L, Zhao S, Ma Z, Gao Y, Zeng L, Luo R, Xie K. Loss of KLF4 and consequential downregulation of Smad7 exacerbate oncogenic TGF-β signaling in and promote progression of hepatocellular carcinoma. Oncogene 2017; 36: 2957-68.

[32] Zhao Q, Cai W, Zhang X, Tian S, Zhang J, Li H, Hou C, Ma X, Chen H, Huang B, Chen D. RYBP expression is regulated by KLF4 and Sp1 and is related to hepatocellular carcinoma prognosis. J Biol Chem 2017; 292: 2143-58.

[33] Li Q, Song W, Wang W, Yao S, Tian C, Cai X, Wang L. Suppression of epithelial-mesenchymal transition in hepatocellular carcinoma cells by Kruppel-like factor 4. Oncotarget 2016; 7: 29749-60.

[34] Burock S, Herrmann P, Wendler I, Niederstrasser M, Wernecke KD, Stein U. Circulating metastasis associated in colon cancer 1 transcripts in gastric cancer patient plasma as diagnostic and prognostic biomarker. World J Gastroenterol 2015; 21: 333-41.

[35] Thakur R, Trivedi R, Rastogi N, Singh M, Mishra DP. Inhibition of STAT3, FAK and Src mediated signaling reduces cancer stem cell load, tumorigenic potential and metastasis in breast cancer. Sci Rep 2015; 5: 10194.

[36] Aigner K, Dampier B, Descovich L, Mikula M, Sultan A, Schreiber M, Mikulits W, Brabletz T, Strand D, Obrist P, Sommergruber W, Schweifer N, Wernitznig A, Beug H, Foisner R, Eger A. The transcription factor ZEB1 (deltaEF1) promotes tumour cell dedifferentiation by repressing master regulators of epithelial polarity. Oncogene 2007; 26: 6979-88.