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Original Article

Expression of MTA2 and Ki-67 in hepatocellular carcinoma and their correlation with prognosis

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Abstract: The aim of this study was to investigate the relationship among MTA2, Ki-67 and HCC patient prognosis. Tissue microarray and immunohistochemistry were used to detect the expression of MTA2 and Ki-67 in HCC samples and corresponding adjacent samples. We found MTA2 and Ki-67 were both increased in HCC tissues than those in adjacent tissues and nuclear MTA2 was associated with Ki-67 ($P = 0.019$). Moreover, nuclear MTA2 was a risk factor of distant metastasis in patients with HCC andKi-67 showed a negative correlation with histological grade ($P < 0.05$, $P < 0.01$, respectively). Multivariate Cox model analysis revealed that Ki-67 expression was an independent prognosis factor in HCC patients ($P = 0.020$). These results indicated there might be a tight correlation among MTA2, Ki-67 and HCC prognosis. MTA2 combined with Ki-67 might be used to predict HCC patient prognosis.

Keywords: MTA2, Ki-67, HCC, liver cancer

Introduction

Liver cancer is one of the most common malignancies, and is responsible for more than 300,000 deaths per annum in China. The majority of diagnosed liver cancer cases are hepatocellular carcinoma. A growing number of studies have found many important proteins, enzymes and genes in liver cancer [1-3].

It's well known that Metastasis is the ultimate cause of death for most cancer patients. MTA2 is one of the members of Metastasis-associated tumor gene family (MTA). It's reported that MTA2 played an important role in distant metastasis of various cancer. In breast cancer, MTA2 enhances metastasis by regulating the Rho signaling pathway [4]. MTA2 also affect prognosis by promoting cell invasion in gastric cancer [5]. Recently, a study found that MTA2 is overexpressed in HCC and its level is associated with tumor size and differentiation in the South Korean population. Additionally, they found patients with high MTA2 expression had a poorer survival than patients with low MTA2 expression [6]. However, related molecular network was still unclear. Several studies had revealed the relationship between MTA2 and Ki-67 in malignant tumors [5, 7]. However, little attention was focused on their relationship in HCC.

Ki-67 is a nuclear antigen present only in proliferating cells. It's one of the most widely used proliferation-associated markers in cancer cells. Numerous studies also found Ki-67 had a close connection with metastasis. Researchers found Ki-67 score was an independent prognostic molecular marker to predict distant metastasis in soft tissue sarcoma [8]. Multivariate regression analysis showed that Ki-67 labeling index in the mucosa adjacent to cancer was the most significant marker for colorectal distant metastasis [9].

Since both MTA2 and Ki-67 had a close connection with metastasis and prognosis, we suspected that MTA2 and Ki-67 might operate together with each other, collaborating in the control of HCC.

Table 1. The expression of MTA2 and Ki-67 in liver tissue

	Cancer tissue	Paracancerous tissue	*P* value
MTA2 (Nucleus)	1.178±0.384	1.000±0.000	0.000
MTA2 (Cytoplasm)	1.278±0.450	1.111±0.316	0.005
Ki-67 (Nucleus)	1.330±0.473	1.000±0.000	0.000

In our study, tissue microarray and immunohistochemical were performed to determine the expressions of MTA2 and Ki-67 in Chinese HCC patients, and analyzed whether any relationships exist among the level of MTA2, Ki-67, pathological variables and prognosis of tumor.

Materials and methods

Clinical sample collection

HCC tissues and adjacent tissues were obtained from the Biobank of National Engineering Center for Biochip at Shanghai. Tissues were quickly fixed with 4% formaldehyde and embedded in paraffin wax. Two clinical pathologists examine tissue samples and full clinical data were obtained. This study was approved by the Ethics Committees of National Engineering Center for Biochip in Shanghai. Informed consent forms were obtained before the operation. We calculated the survival time from the surgery date to the last date of follow-up.

Tissue microarray construction

Tissue microarray was performed according to previous study [10]. Briefly, holes with a 0.6 mm diameters were made to preserve the HCC tissue. Pathologist selected tumor and its adjacent tissues on the paraffin blocks. 0.66 μm serial sections were cut from the arrayed paraffin block and placed into glass slides. At last, the tissue microarray was validated by using HE staining.

Immunohistochemistry

Tissue microarray was removed from the refrigerator and placed in the glass slide for rewarming. Then we heated the tissue microarray at 65°C for about 1 hour to melt away the seal wax. Then, we soaked the slide twice in xylene for 10 minutes, and placed it in absolute alcohol for 8 minutes followed by 95% ethanol for another 5 minutes. Finally, slides were soaked at 70% ethanol for 10 minutes. Antigen retrieval was performed in citrate buffer (pH 6.0) for 30 minutes at room temperature to increase immunoreactivity. Slides were incubated with primary antibody MTA2 (Santa Cruz, sc-55566), Ki-67 (DAKO, IR626) followed incubated with HRP labeled secondary antibody. The intensity of MTA2 and Ki-67 staining was scored as 1 (weak), or 2 (strong). Percentage scores for MTA2 were assigned as 1 (≤ 95%) and 2 (> 95%). Percentage scores for Ki-67 were assigned as 1 (≤ 15%) and 2 (> 15%). A consensus was reached by joint review in case of disagreement.

Statistical analysis

Fisher's exact test and X^2 test were used to compare the variables between groups. Kaplan-Meier was used to compare the survival rates among groups. The Cox proportional hazard model was used for multivariate survival analysis. $P < 0.05$ was regarded as statistically significant. All analyses were carried out by using SPSS 13.0 software.

Results

MTA2 and Ki-67 was highly expressed in HCC tissues

The immunohistochemistry staining results showed that, MTA2 protein was located in the nucleus and cytoplasm. The expression of MTA2 was higher in cancer tissue than that in paracancerous tissues. Nuclear Ki-67 expression was primarily increased in cancer tissues than in paracancerous tissues ($P = 0.000$) (**Table 1**; **Figure 1**).

Relationship among MTA2, Ki-67 and clinic pathological features in HCC

To investigate the relationship between MTA2 and Ki-67, we examined their expression in nucleus and cytoplasm respectively. Nuclear MTA2 expression in cancer tissues was associated with Ki-67 ($r = 0.261$, $P = 0.019$) (**Table 2**).

The results suggested that there were no significant differences between MTA2 expression and sex, age, pathological grade, tumor size, depth of invasion and presence of regional lymph node metastasis. However, there was a correlation between the nuclear MTA2 expression and presence of distant metastasis ($P < 0.05$). Further analysis showed that there was a close relationship between the positive

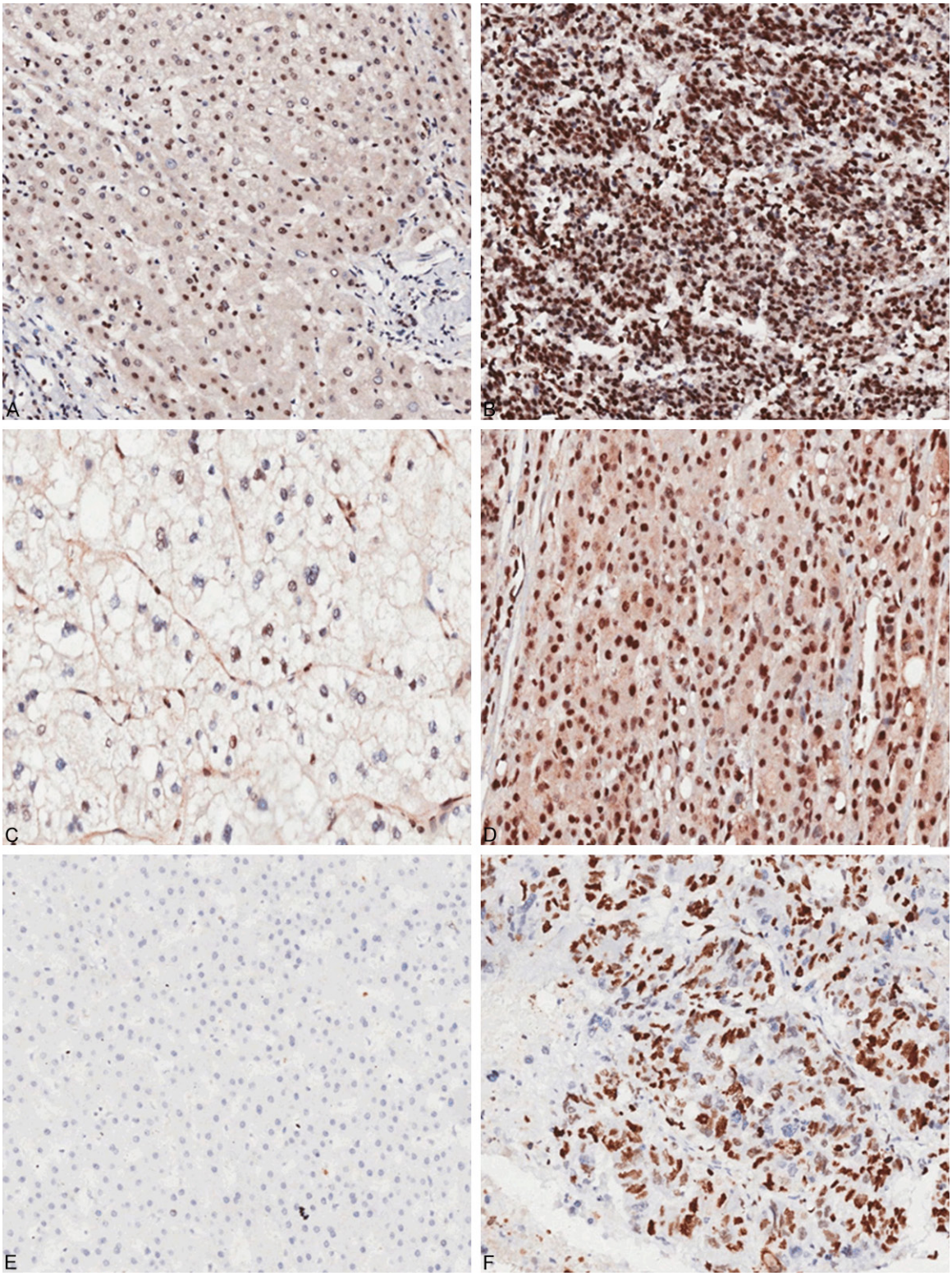

Figure 1. Immunohistochemical analysis of MTA2 and Ki-67 expression in HCC (200×). A. Low expression of nuclear MTA2 in paracancerous tissue. B. High expression of nuclear MTA2 in tumor tissue. C. Low expression of cytoplasmic MTA2 in paracancerous tissue. D. High expression of cytoplasmic MTA2 in tumor tissue. E. Negative expression of nuclear Ki-67 in paracancerous tissue. F. Positive expression of Nuclear Ki-67 in tumor tissue.

Table 2. The relationship between MTA2 and Ki-67

		Nucleus		Cytoplasm		Positive rate of Ki-67 (Cancer)
		MTA2 (Cancer)	MTA2 (Paracancerous)	MTA2 (Cancer)	MTA2 (Paracancerous)	
Nuclear MTA2 (Cancer)	Correlation	1.000	.	.014	-.011	.388**
	P value	.	.	.906	.921	0.001
	N	78	77	77	77	74
Nuclear MTA2 (Paracancerous)	Correlation	.	.	.	.	.
	P value	.	.	.	.	.
	N	77	77	77	77	73
Cytoplasmic MTA2 (Cancer)	Correlation	.014	.	1.000	.148	-.066
	P value	.906	.	.	.198	.577
	N	77	77	77	77	73
Cytoplasmic MTA2 (Paracanerous)	Correlation	-.011	.	.148	1.000	-.096
	P value	.921	.	.198	.	.421
	N	77	77	77	77	73
Positive rate of Ki-67	Correlation	.388**	.	-.066	-.096	1.000
	P value	.001	.	.577	.421	.
	N	74	73	73	73	84

Table 3. The relationship among MTA2, Ki-67 and pathological features

		Age	Pathological grade	Tumor size	T stage	LMN	Metastasis	TNM stage	Sex
Nuclear MTA2 (Cancer)	Correlation	.223	.202	.101	.119	-.083	.242*	.110	.049
	P value	.051	.076	.379	.320	.488	.039	.358	.667
	N	77	78	78	72	72	73	72	78
Nuclear MTA2 (Paracancerous)	Correlation	.	.	.	.	.	.	.	.
	P value	.	.	.	.	.	.	.	.
	N	76	77	77	71	71	72	71	77
Cytoplasmic MTA2 (Cancer)	Correlation	-.126	-.190	.133	-.006	-.099	.	-.006	.078
	P value	.279	.097	.247	.960	.410	.	.960	.499
	N	76	77	77	71	71	72	71	77
Cytoplasmic MTA2 (Paracanerous)	Correlation	-.158	-.133	.161	.108	-.052	.	.108	-.099
	P value	.173	.249	.162	.372	.668	.	.372	.392
	N	76	77	77	71	71	72	71	77
Positive rate of Ki-67	Correlation	.066	.307**	.099	.155	-.108	-.076	.129	.046
	P value	.554	.004	.372	.170	.340	.504	.255	.678
	N	83	84	83	80	80	80	80	84

rate of Ki-67 and pathological grade ($P < 0.01$). However, no significant difference was found between Ki-67 and other clinic pathological features (**Table 3**).

Survival analysis

As the results showed MTA2 was closely related to tumor metastasis and Ki-67, we further examine the role of MTA2 and Ki-67 in overall survival. The Kaplan-Meier survival analysis revealed that survival time for patients with high MTA2 expression in the nucleus was obviously shorter than those with low MTA2 expression ($P = 0.002$). However, cytoplasmic MTA2 expression level had no correlation with survival ($P = 0.851$). Then we analyzed the relationship between Ki-67 and survival time. The positive rate of Ki-67 had a negative correlation with survival rate ($P = 0.001$) (**Figure 2**). We hypothesized that there was a closely connection between these two protein. We divided patients into three groups (Group 1: Ki-67 positive and MTA2 positive, Group 2: Ki-67 negative and MTA2 negative, Group 3: Ki-67 positive/MTA2 negative and Ki-67 negative/MTA2 posi-

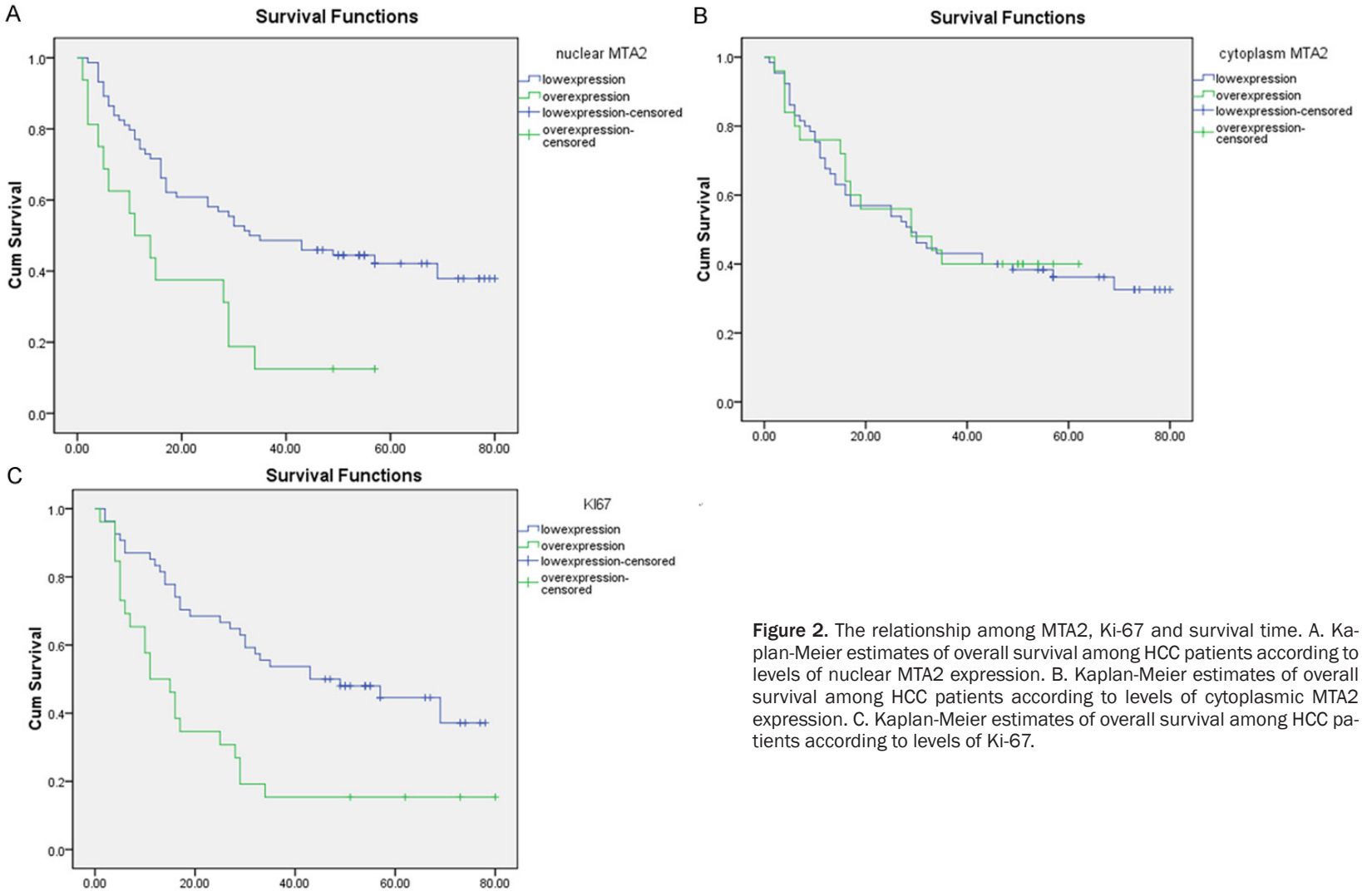

Figure 2. The relationship among MTA2, Ki-67 and survival time. A. Kaplan-Meier estimates of overall survival among HCC patients according to levels of nuclear MTA2 expression. B. Kaplan-Meier estimates of overall survival among HCC patients according to levels of cytoplasmic MTA2 expression. C. Kaplan-Meier estimates of overall survival among HCC patients according to levels of Ki-67.

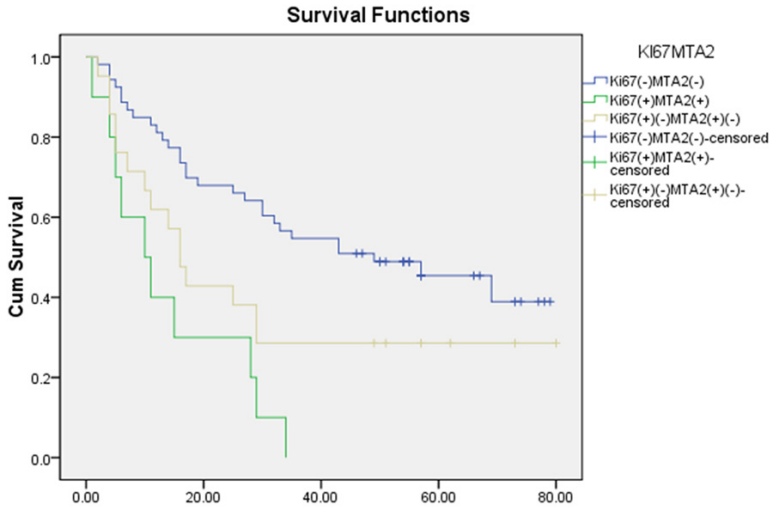

Figure 3. Kaplan-Meier estimates of overall survival among HCC patients according to levels of nuclear MTA2 and Ki-67 expression.

Table 4. Cox regression model for prediction of survival of HCC patients

Features	Multivariable analysis	
	HR (95% CI)	*P* value
Nuclear MTA2 expression	1.899 (0.834-4.321)	0.126
Ki-67 expression	2.224 (1.135-4.357)	0.020
Tumor size	0.899 (0.410-1.970)	0.790
T stage	1.423 (0.432-4.692)	0.562
M stage	14.472 (0.753-278.205)	0.076
TNM stage	1.686 (0.454-6.267)	0.435

tive). Group 1 has the best survival rate. Group 2 has the worst survival rate. Group 3 was medium (P = 0.000) (**Figure 3**).

Single factor analysis indicated that MTA2 expression, Ki-67 expression, tumor size, TNM classification, tumor status and presence of distant metastasis were the primary factors influencing the prognosis of HCC patients. Multivariate Cox model analysis revealed that only Ki-67 expression was an independent prognosis factor in HCC patients (hazard ratio = 2.224, 95% confidence interval 1.135-4.357, P = 0.020) (**Table 4**).

Discussion

As a newly discovered tumor metastasis-related protein family, MTA family is involved in metastasis and prognosis in various cancers. For example, it was reported that MTA2 was negatively associated with HCC prognosis. Nevertheless, this research failed to find relevant genes through which MTA2 affect patient prognosis [11-13]. Some studies have shown MTA2 influence on patient prognosis by interacting with other genes, among which Ki-67 was an important gene involved in cancer cell proliferation and distant metastasis [5, 7]. However, nobody has conducted the relationship between MTA2 and Ki-67 in HCC and joint contributions on HCC.

In the present study, the results showed that both MTA2 and Ki-67 were increased in tumor tissues and nuclear MTA2 expression was positive related to Ki-67 expression (0.019). Then, we found nuclear MTA2 in cancer tissue was related to distant metastasis and Ki-67 was positively correlated with pathological grades. Further Kaplan-Meier survival analysis showed that both MTA2 and Ki-67 had a negative relationship with patient prognosis and combined analysis of positive-MTA2 and positive Ki-67 correlated more highly with overall survival than either positive-MTA2 or positive- Ki-67 alone. Multivariate Cox model analysis revealed that only Ki-67 expression was an independent prognosis factor in HCC patients. We considered that Ki-67 played a critical role in HCC prognosis. Overexpression of Ki-67 might result in poor overall survival of HCC by affecting pathological grades. More probably, overexpression of Ki-67 might lead to poor overall survival of HCC by regulating MTA2 level. We hypothesized thatKi-67 was the most important factor in this regulatory process. Patient with high level of Ki-67 had a higher grade malignancy and worse prognosis. However, whether the regulation mechanism of Ki-67 is dependent of MTA2 or not remains unknown. Recently, researchers had found that Knockdown of MTA2 could significantly reduce the Ki-67 expression and attenuated xenograft growth and lung metastasis [5]. Researchers also found that nuclear MTA2 in lung cancer was associated with Ki-67 expression (r =

0.538, P = 0.000). Ki-67 proliferation index was much higher in nuclear MTA2-positive tumors than in nuclear MTA2-negative tumors. Furthermore, nuclear MTA2 staining had identical distribution to Ki-67 [7].

Proliferation is closely linked to distant metastasis in the development of cancer. The proliferative cancer cells were more likely to attach to vascular endothelium and initiate to metastasis. Ki-67 is primarily expressed in cells at G_1, D and G_2-M phases but not in the G_0 phase of the cell cycle. Since our results showed that the relationship between MTA2 and Ki-67, we hypothesized that MTA2 might play an important role in cancer cell proliferation. To further validate our hypothesis, more cell experiments were needed in the next phase of research. MTA2 and Ki-67 were needed to knockdown respectively in liver cancer cell, and then the mobility and proliferation of cancer cell were needed to examine.

In summary, our study showed that there was a closely relationship among MTA2, Ki-67, and prognosis. NuclearKi-67 expression was associated with the prognosis of HCC patients. Ki-67 is an independent factor to predict the prognosis of HCC patients. Considering our sample size is limited, more cases are required to validate our finding. Moreover, the mechanism of translocation and regulation of MTA2 and Ki-67 in HCC need to be studied.

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Disclosure of conflict of interest

None.

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