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Original Article

Clinical value and function of lncRNA MALAT1 in hepatocellular carcinoma: a comprehensive study with original detection, meta-analysis and *in vitro* verification

Jian-Jun Li[1*], Hong Yang[2*], Xiao-Yong Cai[1], Yu-Bin Huang[1], Si-Qiao Shen[3], Dong-Yue Wen[2], Wei-Jia Mo[3], Zhen-Bo Feng[3], Gang Chen[3], Yi-Wu Dang[3], Dian-Zhong Luo[3]

[1]Department of General Surgery, Western Branch, First Affiliated Hospital of Guangxi Medical University, Nanning 530000, Guangxi Zhuang Autonomous Region, China; Departments of [2]Medical Ultrasonics, [3]Pathology, First Affiliated Hospital of Guangxi Medical University, Nanning 530021, Guangxi Zhuang Autonomous Region, China. [*]Equal contributors.

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Abstract: Background: Hepatocellular carcinoma (HCC) is known as a malignant neoplasm with a high metastasis rate, especially intrahepatic metastasis and poor prognosis. Recent studies have indicated that long noncoding RNAs (lncRNAs) may play an important role in HCC. As the first discovered lncRNA, the metastasis-associated lung adenocarcinoma transcript 1 (MALAT1) has been explored in various cancers. However, the function of MALAT1 in HCC still remains unclear. The aim of this study was to investigate the clinical significance of MALAT1 in HCC with original detection and a meta-analysis, and further to verify the biological functions of MALAT1 *in vitro*. Methods: RT-qPCR was used to examine the expression of MALAT1 in 95 paired HCC cases. A meta-analysis of three available studies was also performed to evaluate the association between MALAT1 expression and clinical features in HCC. In addition, the biological functions on cell growth and apoptosis were investigated with four HCC cell lines (HepG2, SMMC-7221, Hep3B and SNU-449) via different *in vitro* assays. Results: Significantly upregulated MALAT1 was found in the current 95 HCC tissues compared with noncancerous adjacent hepatic tissues. The higher MALAT1 level was remarkably correlated with several clinicopathological parameters, such as tumor nodes (r=0.302, P=0.003), metastasis (r=0.358, P<0.001), TNM stage (r=0.340, P=0.001), and four established markers (nm23, MTDH, p53, VEGF). Meta-analysis further showed that the overexpressed MALAT1 was positively correlated with AFP level (OR=2.37, 95% CI: 1.19-4.73, P=0.002). Additionally, after MALAT1 was silenced in HCC cells, especially in HepG2 and SMMC-7221, the cell growth was suppressed and apoptosis was promoted notably. Conclusion: MALAT1 may play an essential role in the development and deterioration in HCC, in particular in cell growth and apoptosis. Following with the further research, understanding of MALAT1 may make great headway and MALAT1 gains the potential to act as a biomarker in diagnosis, prognosis prediction and targeted therapy in HCC.

Keywords: MALAT1, hepatocellular carcinoma, siRNA, proliferation, apoptosis, caspase

Introduction

Hepatocellular carcinoma (HCC) is one of the most malignant neoplasia in the digestive system and the morbidity rates are the fifth most common type of cancer in men and the seventh in women worldwide, respectively [1, 2]. An abundant of cases is diagnosed in developing countries, which accounts for almost 85% of all cases all over the world [3, 4]. Although surgical resection is the first selection for early stage HCC, the recurrence of tumor cannot be avoided. Additionally, most of cases are diagnosed in the advanced stages. Thus, the prognosis of HCC is dismal, and it maintains low rates of the 5-year survival, despite the advancement in diagnosis and multidisciplinary approaches [5-7]. Since the underling mechanism of HCC remains unclear, it is necessary and essential to explore the cause and mechanism of tumorigenesis and progression of HCC.

Recently, long noncoding RNAs (lncRNAs) have become a hotspot, which contain longer than

200 nt [8-11]. However, only a few of lncRNAs have been thoroughly explored. The metastasis-associated lung adenocarcinoma transcript 1 (MALAT1), one of the first lncRNAs discovered and initially identified in 2003 [12], later also known as nuclear-enriched abundant transcript 2 (NEAT2), has been reported to be widely overexpressed in some malignancies [13, 14]. Furthermore, MALAT1 can assist to predict metastasis and unfavorable prognosis [13, 15-20]. MALAT1 has also been reported to act as an important metastasis-relevant oncogene, which was overexpressed and related with the rate of tumor recurrence in HCC [21]. Besides, Luo et al. found that the expression of MALAT1 was markedly elevated in hepatoblastoma (HPBL) tissues than HCC [22]. However, only two findings with a small sample size (n=60 and n=32) explained the characteristics of MALAT1 in HCC [21, 23]. Thus, we carried out this study to investigate the clinical significance of MALAT1 in HCC tissues via direct detection and a meta-analysis, and further to verify its biological functions *in vitro*. The expression of MALAT1 was detected in 95 HCC tissues and their paired normal adjacent tissues (NATs) by real-time RT-qPCR. A meta-analysis was then performed to summarize the relevance of MALAT1 expression and HCC. Furthermore, the function on proliferation and apoptosis of MALAT1 was investigated by a series of experiments *in vitro*. Through this comprehensive study with 95 pairs of HCC tissue, a meta-analysis and *in vitro* verification, we expected to provide a further understanding of the clinical value and function of MALAT1 in HCC.

Materials and methods

Clinical samples

To detect the level of MALAT1 in HCC tissues and paired NATs, 95 paraffin-embedded tissues from patients with HCC, aged from 29 to 82 years, were collected in the First Affiliated Hospital of Guangxi Medical University (Nanning, Guangxi, China) between March 2010 and December 2011. All of the patients underwent none of chemotherapy or radiotherapy before surgery. The histopathological diagnosis was determined according to the World Health Organization criteria, and all of the samples were diagnosed and reviewed by three pathologists individually (DL, ZF and GC). This study was approved by the Medical Ethical Committee of the First Affiliated Hospital of Guangxi Medical University. All patients provided the informed consent for clinical research. The clinicopathological characteristics were described in **Table 1**.

RT-qPCR

In vitro, the RNA isolation and RNA normalization, as well as cDNA synthesis were described previously [24, 25]. The extracted RNA concentration and quality were detected by Nanodrop 2000. Real-time qPCR for MALAT1 was performed with Applied Biosystems PCR7900. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was detected as an endogenous control. The MALAT1 abundance in each sample was normalized to its reference GAPDH. Each experiment was performed in triplicate. The lncRNA expression levels were calculated by $2^{-\Delta cq}$. Primers sequences were listed as below: MALAT1: Forward: 5'-GGATCCTAGACCAGCATGCC-3', Reverse: 5'-AAAGGTTACCATAAGTAAGTTCCAGAAAA-3'. GAPDH: Forward: 5'-GTAAGACCCCTGGACCACCA-3', Reverse: 5'-CAAGGGGTCTACATGGCAACT-3'.

Meta-analysis to explore the clinical role of MALAT1 in HCC

We selected literatures by retrieving PubMed, Web of Science, EMBASE, Science Direct, Google Scholar, Wiley Online Library, Chinese CNKI, Wan Fang, China Biology Medicine disc and Chong Qing VIP. The key words were followed medical subject headings (MeSH): ("HCC" or "hepatocellular carcinoma" or "hepatic carcinoma" or "liver cancer") and ("MALAT1" or "MALAT-1" or "metastasis associated lung adenocarcinoma transcript 1" or "NEAT2"). Those eligible studies were accorded with inclusion criteria as following: (1) The study explored the association between the expression level of MALAT1 and clinicopathological characteristics in HCC tissues; (2) Expression of MALAT1 was detected by RT-qPCR; (3) Publications were in Chinese and English. The exclusion criteria included: (1) Confusing and unclear data; (2) Overlapping or irrelevant studies (animal studies, review, or case report). The following data was extracted from the studies: first author's names, publication year, language, sample size, method of MALAT1 detection, patient characteristics, and MALAT1 expression level. Statistical heterogeneity

Table 1. Association of MALAT1 expression with clinicopathological parameters in HCC

Clinicopathological Feature		n	MALAT1 relevant expression ($2^{-\Delta Cq}$)		
			Mean ± SD	t	P
Tissue	Adjacent non-cancerous liver	95	1.8553 ± 0.8951	-4.368#	<0.001
	HCC	95	2.4186 ± 0.8826		
Age	≥50	46	2.4000 ± 0.9409	-0.198	0.843
	<50	49	2.4361 ± 0.8336		
Gender	male	75	2.4231 ± 0.8542	0.094	0.925
	female	20	2.4020 ± 1.0060		
Differentiation	high	6	2.4000 ± 0.3899	F=0.25*	0.779
	moderate	60	2.4662 ± 0.8551		
	low	29	2.3241 ± 1.0165		
Size	<5 cm	18	2.4667 ± 0.9274	0.255	0.799
	≥5 cm	77	2.4074 ± 0.8778		
Tumor nodes	single	52	2.1777 ± 0.7956	-3.053	0.003
	multi	43	2.7100 ± 0.9034		
Metastasis	Without metastasis	46	2.0943 ± 0.7795	-3.696	<0.001
	With metastasis	49	2.7231 ± 0.8721		
Clinical TNM stage	I~II	22	1.8745 ± 0.8662	-3.489	0.001
	III~IV	73	2.5826 ± 0.8249		
Portal vein tumor embolus	-	63	2.3768 ± 0.9352	-0.646	0.52
	+	32	2.5009 ± 0.7762		
Vaso-invasion	-	59	2.3837 ± 0.8568	-0.491	0.624
	+	36	2.4758 ± 0.9330		
Tumor capsular infiltration	With complete capsule	45	2.2489 ± 0.7375	-1.799	0.075
	No capsule or infiltration	50	2.5714 ± 0.9776		
HCV	-	63	2.4265 ± 0.8227	0.121	0.904
	+	32	2.4031 ± 1.0040		
HBV	-	17	2.5353 ± 0.8455	0.599	0.55
	+	78	2.3932 ± 0.8938		
AFP	-	41	2.3585 ± 0.8170	-0.274	0.785
	+	38	2.4139 ± 0.9779		
Cirrhosis	-	50	2.3308 ± 09719	-1.023	0.309
	+	45	2.5162 ± 0.7707		
nm23	-	20	1.9370 ± 0.9929	-2.849	0.005
	+	75	2.5471 ± 0.8106		
MTDH	-	50	2.2308 ± 0.8726	-2.681	0.009
	+	39	2.7264 ± 0.8559		
P53	-	40	2.1210 ± 0.7990	-2.912	0.004
	+	55	2.6351 ± 0.8841		
P21	-	62	2.3265 ± 0.9118	-1.402	0.164
	+	33	2.5918 ± 0.8100		
VEGF	-	25	2.0656 ± 1.0274	-2.116	0.042
	+	70	2.5447 ± 0.7955		
Ki-67 LI	Low	47	2.3030 ± 0.9102	-1.268	0.208
	High	48	2.5319 ± 0.8490		
MVD	Low	47	2.3753 ± 0.9856	-0.471	0.639
	High	48	2.4610 ± 0.7770		

#paired *t* test; *ANOVA was used to analyze the differentiation. LI: labelling index, MVD: micro-vessel density.

among the studies was tested by inconsistency (I^2) and Q test (Chi-squared test; χ^2). STATA 12.0 (STATA Corp., College, TX) was used to perform statistical analysis. All *P* values were tested by two-sided, and P<0.05 was considered as statistically significant.

Cell lines and transfection

The human HCC cell lines HepG2, SMMC-7221, Hep3B, SNU-449 were purchased from the American Type Culture Collection (ATCC, Netherlands), and cultured in Roswell Park Memorial Institute (RPMI)-1640 with high glucose (Invitrogen Corp., Belgium) supplemented with 10% heat-inactivated fetal bovine serum (FBS; Perbio Science NV, Belgium), 1 mM L-glutamine and 1 mM Penicillin-Streptomycin Solution at 37°C in a humidified incubator with 5% CO_2.

SiRNA transfection

The MALAT1 specific small interfering RNAs (siRNAs) were designed according to literatures and four siRNAs were pooled together for the knock-down experiments. The sequences were as following: MALAT1-1, 5'-GAGGUGUAAAGGGAUUUAUTT-3' (3099-3117) [26]; MALAT1-2, 5'-GGCAAUGUUUUACACUAUUTT-3' (6294-6312) [27]; MALAT1-3, 5'-GCAGAGGCAUUUCAUCCUU-3' (6368-6386)[28]; MALAT1-4, 5'-CACAGGGAAAGCGAGUGGUUGGU-3' (2982-3004) [29]. All four cell lines were plated in 24-well plates (2.5×104 cells per well) and cultured for 24 h, then transfected with siRNA by CombiMAGnetofection (OZ BIOSCIENCES, Marseille cedex 9 France), the final volume was 500 μl RPMI-1640 including 10% serum without antibiotics. After being transfected for 96 h, the cells were collected to RT-qPCR and other analyses.

Cell growth

Cells were seeded in 96-well plates at a density of 2500 cells/well in a volume of 200 μl per well, cultured overnight and transfected with MALAT1-siRNA. Then the plates were incubated at 37°C with 5% CO_2 for 24 h, 48 h, 72 h and 96 h, respectively. MTS (20 μl, CellTiter96 AQueous One Solution Cell Proliferation Assay G3580, Promega, Madison, WI, USA) was added to each well. After the cells were incubated for 2 h at 37°C, the absorbance at 490 nm was measured with a microplate reader (Scientific MultiskanMK3, Thermo Finland). The detailed methods were described previously [30-33].

Cell viability

Cell viability was further confirmed by another assay of fluorimetric detection of resorufin (CellTiter-Blue Cell Viability Assay, G8080, Promega, Madison, WI, USA) according to the manufacturer. The treatment was described previously [30-33]. Fluorimetry was detected by an FL600 fluorescence plate reader (Bio-Tek, Winooski, Vermont, USA).

Caspase-3/7 activity detection

Caspase-3/7 activity was tested by a synthetic rhodamine labeled caspase-3/7 substrate (Apo-ONE® Homogeneous Caspase-3/7 Assay, G7790, Promega, Madison, WI, USA), immediately after the cell viability was detected. Cells were maintained at room temperature for 1 h, the fluorescence was measured by a FL600 fluorescence plate reader (Bio-Tek, Winooski, Vermont, USA). The procedures were described previously [30-33].

Cell apoptosis and nuclear morphology

The cells in plates were washed with cold PBS, then stained with Hoechst 33342 (1 mg/ml, Sigma-Aldrich N.V. Belgium) and propidium iodide (PI) (1 mg/ml, Sigma-Aldrich N.V. Bornem, Belgium) for 15 min. The cells were subsequently observed under an advanced fluorescence microscope (ZEISS Axiovert 25, Belgium). Condensation of nuclear fragmentation and its chromatin were judged by the standard of apoptosis and nuclear chromatin. The specific standards and methods were described previously [30-33].

Statistical analysis

All the statistical analysis was performed with SPSS 20.0. Data were presented as mean ± SD. The difference between expression of MALAT1 in HCC tissues and NATs was tested by Paired Student's T-test. The difference of expression of MALAT1 between subgroups of HCC with different clinicopathological parameters was applied by unpaired Student's T-test. One-way analysis of variance (ANOVA) test was performed to examine the difference of MALAT1 in the differentiation which was divided into 3 groups. The relationship between MALAT1

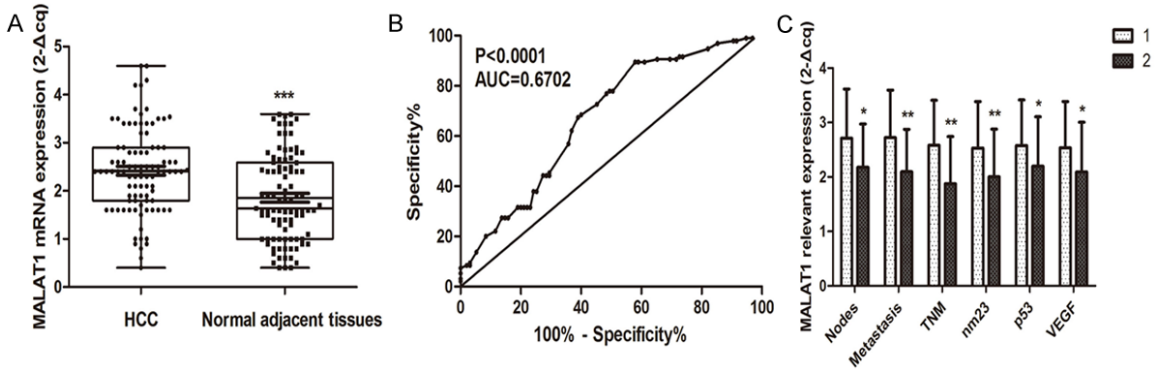

Figure 1. The level of MALAT1 analyzed by RT-qPCR in HCC tissues. A. MALAT1 expression was higher in HCC compared with normal adjacent tissues. ***P<0.001. B. ROC curves evaluated MALAT1 in HCC. The AUC of MALAT1 was 0.6702. C. The relationships between MALAT1 and clinicopathological features. Nodes: 1. multiple nodes, 2. single node; Metastasis: 1. with metastasis; 2. without metastasis; TNM: 1. TNM stage III-IV 2, TNM stage I-II; nm23: 1. positive (+), 2. negative (-); p53: 1. high level (+), 2. low level (-); VEGF: 1 minus (+), 2 plus (-). *P<0.05; **P<0.01, ***P<0.001.

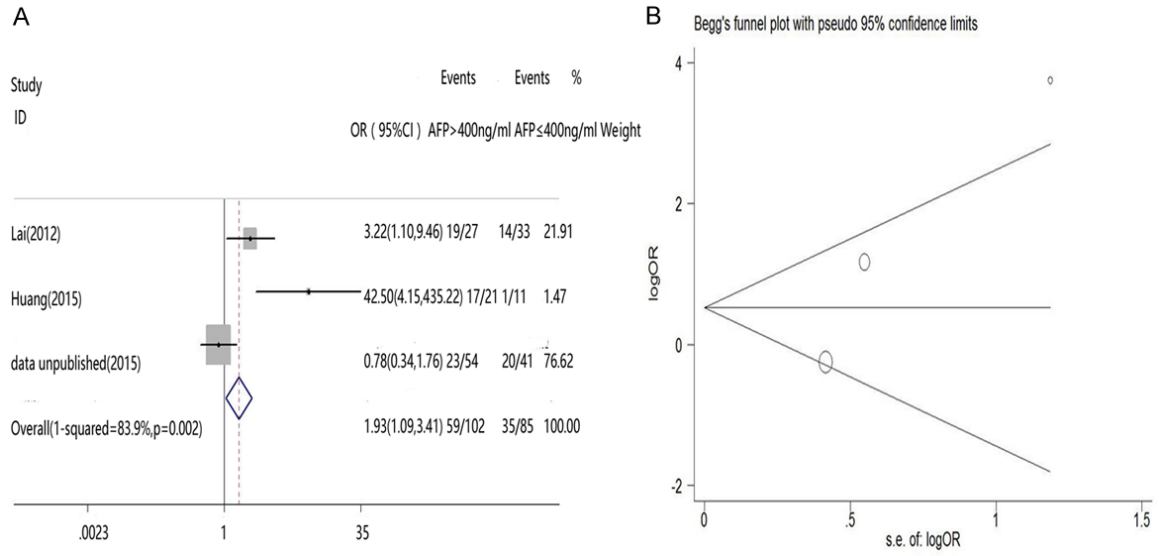

Figure 2. Cells morphologic change by Hoechst 33342 and PI double fluorescent staining assay. In the group of MALAT1 siRNA, nuclear chromatin condensed and fragile, and the late apoptotic cells were stained with red fragmentations.

expression and clinicopathological parameters was tested with Spearman rank correlation. ROC curve was applied to estimate the diagnostic value. GraphPad Prism 5.0 was applied to plot all graphs. P<0.05 (2-sides) was considered statistically significant.

Results

Detection of MALAT1 in HCC tissues by RT-qPCR

MALAT1 was significantly overexpressed in HCC tissues compared with NATs (2.4186 ± 0.8826 vs 1.8553 ± 0.8951, P<0.001; **Figure 1A**). The diagnostic value of MALAT1 for HCC was proved by ROC curve, and the area under curve (AUC) was 0.6702 (95% CI 0.5934-0.7471, P<0.0001). The cut-off value was 1.595, as extracted from ROC. The high sensitivity was 89.47% (**Figure 1B**).

Relationships between MALAT1 expression and clinical parameters in HCC tissues

To investigate the correlation between MALAT1 expression and clinicopathological features, the difference of its expression in subgroups of

Table 2. Characteristics of all studies in the meta-analysis

First author	Year	Regions	Language	Sample size (F/M)	Cancer type	Method
Lai et al	2012	China	English	60 (5/55)	HCC	RT-qPCR
Huang et al	2015	China	English	32 (2/30)	HCC	RT-qPCR
The current data	2015	China	English	95 (20/75)	HCC	RT-qPCR

F: female; M: male; HCC: hepatocellular carcinoma; RT-qPCR: quantitative real-time polymerase chain reaction.

HCC with diverse clinicopathological features was tested by Student's *t*-test analysis and strong relevancy was found. The level of MALAT1 in HCC with multi-tumor nodes was higher than that in the group of single tumor node (2.7100 ± 0.9034 vs 2.1777 ± 0.7956, P=0.003). MALAT1 level in HCC patients with metastasis increased pronouncedly than those without metastasis (2.7231 ± 0.8721 vs 2.0943 ± 0.7795, P<0.001). The expression of MALAT1 in the clinical III-IV stage was prominently upregulated by comparison with that in the stage I-II (2.5826 ± 0.8249 vs 1.8745 ± 0.8662, P=0.001). Moreover, we also detected the correlation of MALAT1 with several well-established biological markers expression. Level of MALAT1 was much higher in nm23 positive group than that in negative group (2.5471 ± 0.8106 vs 1.9370 ± 0.9929, P= 0.005). MALAT1 expression increased strikingly in HCC with high MTDH level compared with low MTDH (2.7264 ± 0.8559 vs 2.2308 ± 0.8726, P=0.009). MALAT1 displayed a higher expression with high-level p53 than low-level p53 (2.6351 ± 0.8841 vs 2.1210 ± 0.7990, P=0.004). In comparison with positive VEGF, the expression of MALAT1 was reduced in negative VEGF group (2.5447 ± 0.7955 vs 2.0656 ± 1.0274, P=0.042) (**Table 1**; **Figure 1C**). However, the expression of MALAT1 had no significant relationship with other features, such as age, gender, tumor size, portal vein tumor embolus, vaso-invasion, tumor capsular infiltration, cirrhosis and so on (**Table 1**).

Meta-analysis

Ultimately, three eligible literatures were adopted, including the current data, studies by Lai et al and Huang et al [21, 23]. MALAT1 overexpression was significantly related with AFP level (OR=2.37, 95% CI: 1.19-4.73, P=0.002) in total 187 tissues (**Figure 2A**). Begg's and Egger's test were applied to judge the publication bias. The two tests reveal no publication bias in the subgroup of AFP (Begg's test: P= 0.602, Egger's test: P=0.663. **Figure 2B**; **Table 2**). Unfortunately, the level of MALAT1 showed no relevance to metastasis, stage of TNM, tumor size and tumor nodes in the meta-analysis.

Function of MALAT1 siRNA on malignant phenotypes of HCC cells

MTS was applied to study the effect of MALAT1 on proliferation of HCC cell lines (HepG2, SMMC-7221, Hep3B, SNU-449). As shown in **Figure 3A**, we confirmed that cell growth was inhibited with MALAT1 knock-down in all four cell lines. HepG2 was the most sensitive cell line with its cell proliferation ability being decreased to 51% ± 6.25% (P<0.001) 96 h post-transfection. Fluorimetric resorufin viability assay was assessed to verify the accuracy of the MTS results (**Figure 3B**). The results showed that the viability in all 4 transfected cell lines was decreased, and the largest reduction occurred in HepG2 (50% ± 6.08%, P<0.001) 96 h post-transfection. The effect of MALAT1 on cell growth inhibitor was also confirmed by using Hoechst 33342 and PI double staining assay (data not shown).

For the sake of attesting the function of MALAT1 siRNA on cell apoptosis, fluorescent caspase-3/7 assay was then employed. The results displayed a time-dependent activity of caspase-3/7 activity in 4 cell lines (**Figure 4A**). The most sensitive cell line was SMMC-7221 (4.44 ± 0.40, P<0.001, 96 h post transfection). The effect of MALAT1 siRNA on apoptosis was also assessed with Hoechst 33342 and PI double staining assays. A significant higher apoptosis rate was found in HepG2 (96 h, 3.55 ± 0.19, P<0.001). Most remarkable late apoptotic cells and necrotic cells were examined in the group of MALAT1 siRNAs in four cell lines at 96 h (HepG2: 3.55 ± 0.19, P<0.001; SMMC-7221: 3.81 ± 0.22, P<0.001; Hep3B: 3.66 ± 0.43, P<0.001; SNU449: 2.78 ± 0.57, P<0.001, **Figures 4B**, **5**).

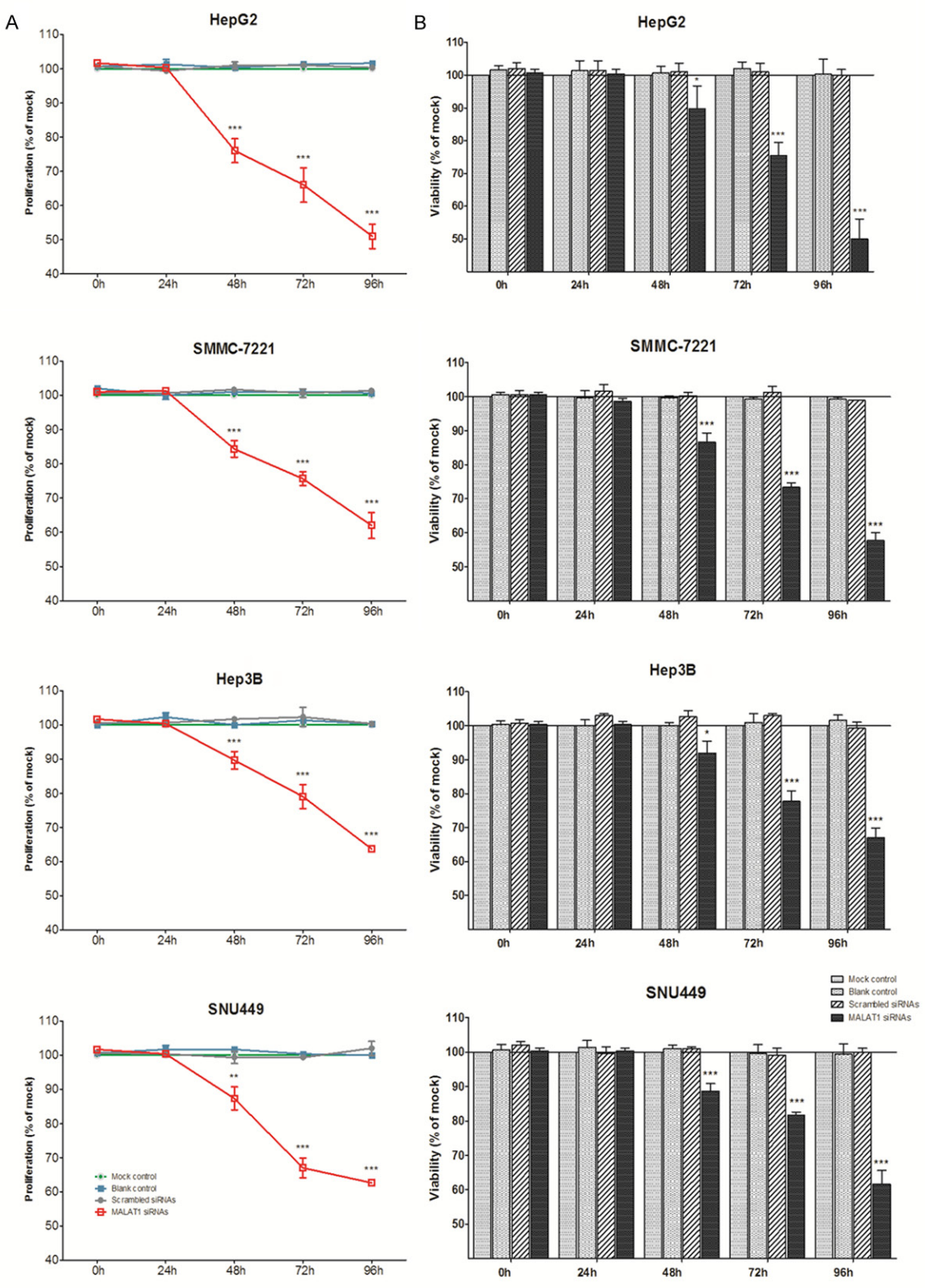

Figure 3. Forest plot and funnel plot for meta-analysis. A. Forest plot of the correlation between MALAT1 expression in positive AFP (AFP>400 ng/mL) and negative AFP (AFP≤400 ng/mL). B. Funnel plot of publication bias about MALAT1 and AFP.

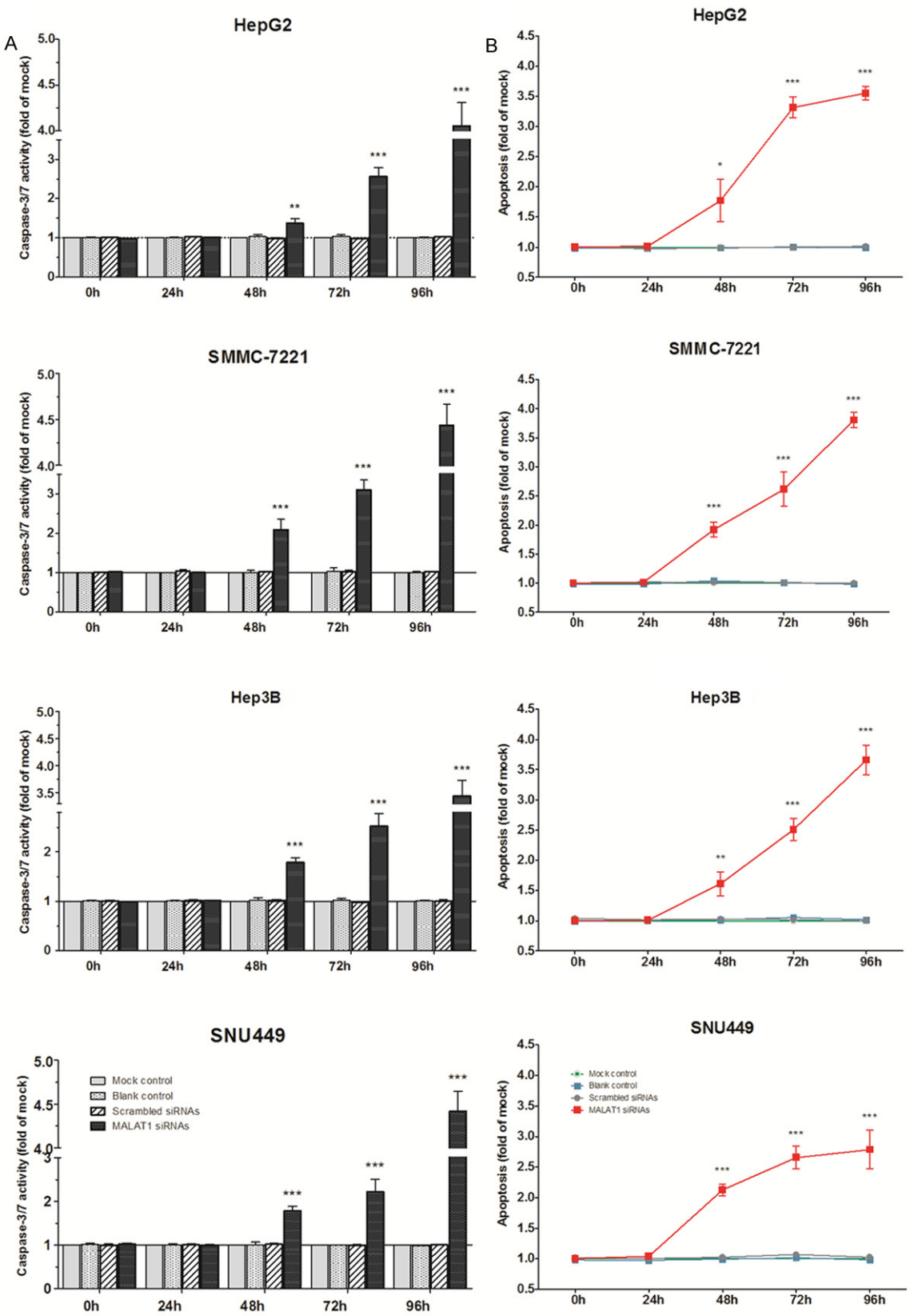

Figure 4. Effect of MALAT1 siRNA on the growth of HCC cell lines. A. A time-dependent function was probed by MTS. Cells proliferation were significantly inhibited in 96 h. B. MALAT1 siRNA remarkably reduced the cells viability. *P<0.05; **P<0.01; ***P<0.001.

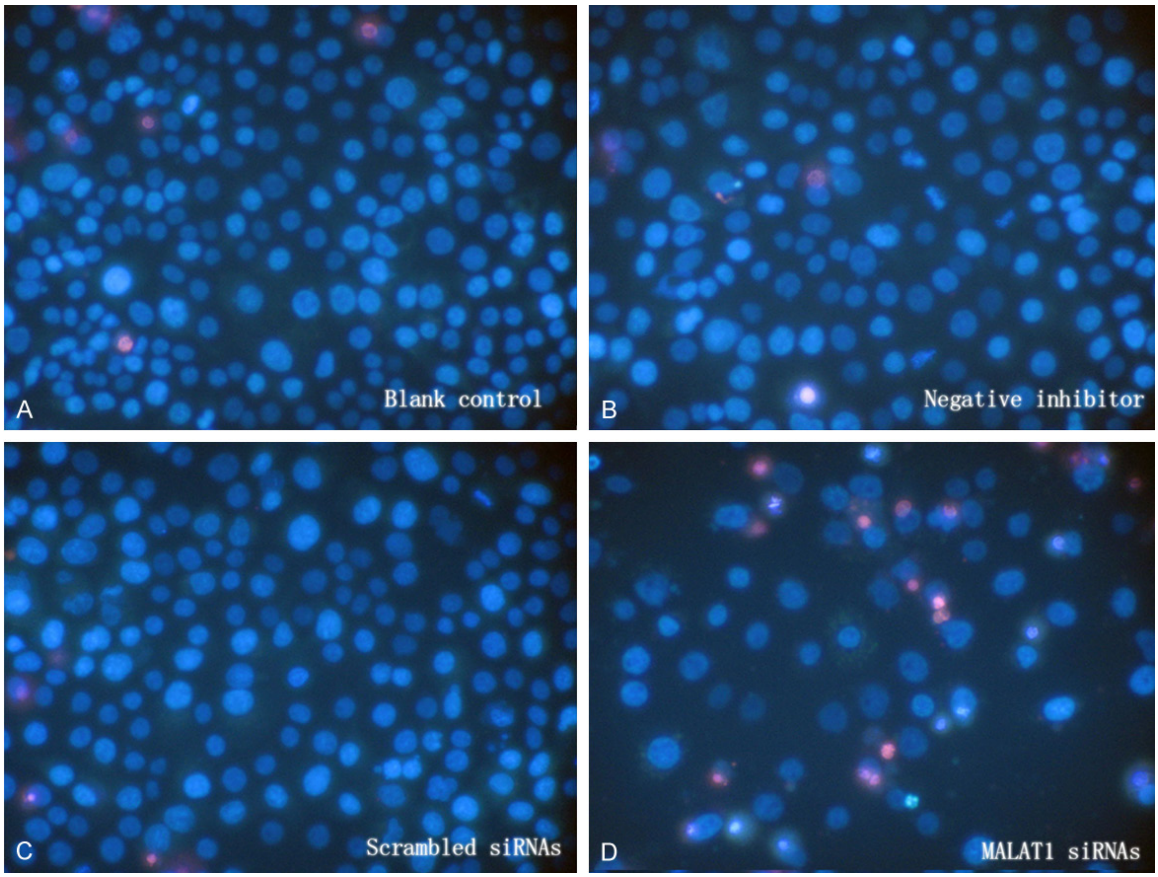

Figure 5. Apoptosis of HCC cells. A. Blank control, B. Negative inhibitor, C. Scrambled siRNAs, D. MALAT1 siRNAs (Hoechst 33342 and PI double staining assay).

Discussion

In recent years, a variety of studies have indicated that the abnormal expression of lncRNAs is closely related to the occurrence and development of tumor. MALAT1, as the first discovered lncRNA, has been reported to be involved in the metastasis and progression in various types of carcinomas, such as lung cancer, osteosarcoma, breast cancer , and glioma in several meta-analysis [13, 14, 17-20, 26, 29]. Not surprisingly, MALAT1 has been also found to be involved in the formation of HCC [21, 23]. Based on previous reports, it is suggested that MALAT1 may be an oncogene, which plays a vital role in the hepatocarcinogenesis and development of HCC. Liu et al found that 659 lncRNAs were differentially expressed in HCC microarray by comparison with NATs (171 were downregulated and 488 were upregulated). The expression of MALAT1 was detected in HCC tissues and matched adjacent non-tumor tissues (n=29). However, no significant statistical difference was found [34]. The potential reason of negativity in Liu's study may be the insufficiency of the sample size. Overexpression of MALAT1 was also reported to be associated with recurrence after liver transplantation [21]. But the clinical role and function of MALAT1 in HCC remains largely unknown. Thus, in the study, we made great efforts in determining the role of lncRNA MALAT1 in HCC tissues by exploring its relationships with clinical characteristics, conducting a meta-analysis and performing *in vitro* validated experiments.

We first corroborated the upregulation of MALAT1 and its role as an oncogene in 95 cases of HCC tissues by real time RT-qPCR, and it supports that MALAT1 might play an essential role in the tumorigenesis of HCC. MALAT1 could also be detected in peripheral blood mononuclear cells with some other malignancies, such as pancreatic cancer [35] and lung cancer [36, 37]. Even no report has been available on the detection of circulating MALAT1 in

HCC, it can largely be speculated that MALAT1 could assist the early detection of HCC, which needs further verification in the future. In the current study, we also found that there was also a close relationship between MALAT1 expression and a few clinicopathological parameters related to the disease deterioration and metastasis of HCC. Lai et al [21] investigated that the patients with elevated MALAT1 expression had a lower 3-year cumulative disease-free survival rate. Unfortunately, we did not achieve the expected relationship between MALAT1 and prognosis of HCC, probably due to the shortness of the follow-up time. In the previous meta-analyses on the MALAT1 [13, 15-20, 29], the roles of MALAT1 in all tumors were summarized. These studies fully illustrated the relationship between the high level of MALAT1 and poor overall survival (OS). The available meta-analyses [13, 16, 17] only demonstrated that upregulated of MALAT1 was associated with two parameters: tumor size or metastasis of lymph nodes. But these studies still did not explain the association between MALAT1 and other clinical parameters. We are the first to verify the relationship between MALAT1 and the clinicopathological parameters, particularly in HCC by meta-analysis. Despite the fact that our meta-analysis has only found the significant correlation between MALAT1 and AFP, this research still manifests the close relationship between MALAT1 and HCC, since AFP has been widely used as the only marker of HCC in clinical management in Asia. Nevertheless, the other clinical features, such as tumor nodes, metastasis and clinical TNM stage, showed no statistically significance. However, the limitation and bias can not be ignored in the current meta-analysis, since only three studies were included.

To further explore the biological function of MALAT1 in HCC, we investigated its role in 4 cell lines through siRNA transfection to silence MALAT1. The results suggested that HCC cell proliferation was inhibited and cell apoptosis was reduced after MALAT1 was knocked down. Above all, high expression of MALAT1 was correlated with proliferation and apoptosis, which was consistent with the study of Lai et al [21]. According to other studies and the experimental results of our own, we can infer that MALAT1 might regulate the development of HCC by influencing the growth and apoptosis of HCC cells.

However, the specific mechanism of MALAT1 in HCC has not been clarified. We examined the relationship between MALAT1 and some tumor related markers, which is a distinctive part of our study from Lai's [21]. As is known, oncogene nm23 is involved in tumor metastasis and MTDH plays a key role in metastasis as well. P53 is a tumor suppressor and inhibits cancer development, and the role of VEGF is to promote tumor angiogenesis and development. These genes are of paramount importance in the occurrence and development of HCC. MALAT1 might be bound up with the tumor progression related to the malignant biological behavior by affecting the expression and activity of these markers in HCC. Besides, the interaction effect between MALAT1 and microRNAs (miRNAs) was widely studied. MALAT1 bound with miRNA could therefore regulate the tumor development. MALAT1 can be tethered with miR-1 to exert the oncogenic function in breast cancer [38]. Moreover, the posttranscriptional regulation of MALAT1 by miR-1/slug axis in nasopharyngeal carcinoma [39], miR-125b/SIRT7 axis in bladder cancer [40] and miR-200s/ZEB2 in clear cell kidney carcinoma [41] were also reported. In addition, we previously reported that the transcription factor Sp1/3 could regulate MALAT1 expression in HCC cells through *in vitro* experiments [23]. Ji et al [26] and Dong et al [42] confirmed that MALAT1 could regulate cells metastasis via Wnt/β-Catenin signal pathway in colorectal cancer and PI3K/Akt pathway in osteosarcoma, respectively. These two pathways are involved in tumor formation and progression. MALAT1 loss resulted in a reduction of branching morphogenesis in MMTV-PyMT- and Her2/neu-amplified tumor organoids, improved cell adhesion, and loss of migration in a mouse mammary carcinoma model. MALAT1 knockdown led to alterations in gene expression and changes in splicing patterns of genes involved in differentiation and protumorigenic signaling pathways in this mouse mammary carcinoma model [43]. Up-regulation of MALAT1 in human neuroblastoma cells under hypoxic conditions could enhance fibroblast growth factor 2 (FGF2) expression and promote vasculature formation, and thus plays an essential role in tumor-driven angiogenesis [44]. Most recently, MALAT1 was report to function to promote cervical cancer invasion and metastasis via induction of epithelial-mesenchymal transition

(EMT) [45]. Therefore, the relationship among MALAT1, miRNAs and other genes in some key pathways may form a circular adjustment, and play a more effective role in regulating the tumor, including HCC.

Conclusions

Our findings indicate that MALAT1 plays a crucial role in the carcinogenesis and deterioration of HCC, partially due to the influence on tumor cell growth and apoptosis. Furthermore, MALAT1 gains the potential to become a biomarker in diagnosis, prognosis prediction and targeted therapy in HCC. However, the underlying molecular mechanism of MALAT1 has not been well established yet, more convincing studies are needed for further investigations.

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Disclosure of conflict of interest

None.

Address correspondence to: Yi-Wu Dang and Dian-Zhong Luo, Department of Pathology, First Affiliated Hospital of Guangxi Medical University, 6 Shuangyong Road, Nanning 530021, Guangxi Zhuang Autonomous Region, China. Fax: 0086-771-5356534; E-mail: dangyiwu@126.com (YWD); 13878802796@163.com (DZL)

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