

Original Article

Upregulation of MALAT1 expression predicts a poor prognosis in the development of intracranial aneurysm (IA)

Yian Ouyang, Yugang Jiang, Mengqiang Yu, Mingming Zhang, Zhigang Tan

Department of Neurosurgery, Second Xiang-Ya Hospital of Central South University, Changsha, Hunan Province, People's Republic of China

Received February 10, 2017; Accepted February 25, 2017; Epub May 1, 2017; Published May 15, 2017

Abstract: The aim of this study was to detect the role of serum MALAT1 expression levels in patients of intracranial aneurysm (IA) and identify its correlation with the development of IA. The 105 IA blood samples were collected from peripheral vein, and the control group was 40 healthy volunteers. Real-time PCR analysis was performed to detect the expression of MALAT1. Kaplan-Meier survival curves and long-rank test were used for survival analysis. Univariate and multivariate Cox proportional hazards regression analysis was performed to analyze the prognosis for intracranial aneurysm (IA) patients. In the study, our results showed that the MALAT1 expression in intracranial aneurysm (IA) patients was significantly higher MALAT1 expressions than that in the healthy volunteers. Furthermore, we found that MALAT1 expression was closely associations with hypertension history, rupture and Hunt-Hess level. Patients with higher MALAT1 expression had short disease-free survival (DFS) and poor overall survival (OS) than those with lower MALAT1 expression. Univariate and Multivariate Cox proportional hazards regression analysis revealed that MALAT1 expression, hypertension history, rupture and Hunt-Hess were independent risk factors for predicting the IA patient prognosis. Thus, our results demonstrated that serum MALAT1 expression in IA patients may be a novel predicted biomarker for prognosis.

Keywords: Intracranial aneurysm, MALAT1, long non-coding RNA, prognosis

Introduction

Intracranial aneurysms (IA) presents cerebrovascular lesions characterized by weakening and abnormal dilatation of cerebral arteries and therefore putting the aneurysm at risk for rupture [1]. Intracranial aneurysms (IA) occur in about 0.4-6% of the population worldwide and approximately 0.7%-1.9% IA will rupture. Some risk factors for IA are positive family history, increasing age, hypertension, and smoking and the rupture results in subarachnoid hemorrhage (SAH) [2, 3]. If IAs are diagnosed and surgically treated before rupture, the mortality rates significantly decrease to as low as 0-2.5% [4, 5]. Therefore, it is important to investigate novel biomarker to improve patient management.

Abnormally expressed long non-coding RNA (lncRNA) might be associated with disease pro-

gression. Non-coding RNAs acts as novel biomarkers in tumor prognosis in patients [6]. lncRNA-uc003wbd and lncRNA-AF085935 were observed with an aberrant serum level in HCC and HBV patients, which is showing that both lncRNA-uc003wbd and lncRNA-AF085935 are able to be potential biomarkers for HCC and HBV screening [7]. Plasma POU3F3 could serve as a potential biomarker for diagnosis of ESCC, and the combination of POU3F3 and SCCA was more efficient for ESCC detection, in particular for early tumor screening [8]. Circulating CUDR, LSINCT-5 and PTENP1 signature in serum was identified as diagnostic marker for GC [9]. High expression of circulating serum lncRNA RP11-445H22.4 in breast cancer patients: a Chinese population-based study showed a remarkable improvement compared with the clinical serum carcinoembryonic antigen with estrogen receptor (ER), progesterone receptor (PR) [10]. Serum long non-coding RNA, snoRNA host gene 5 level

Higher MALAT1 is a poor prognosis for intracranial aneurysm (IA)

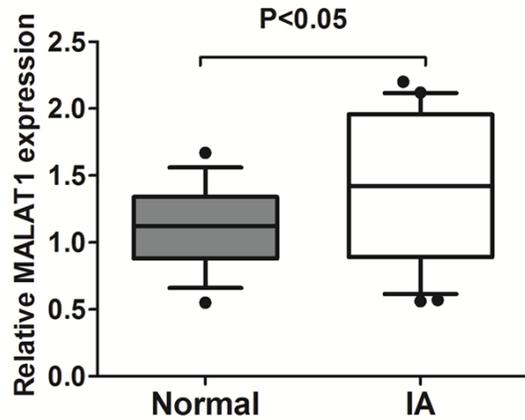


Figure 1. MALAT1 was upregulated in IA patient blood samples. QRT-PCR was performed to analyze the MALAT1 expression in 105 IA patient blood samples compared with the control group. ** $P < 0.05$.

acted as a new tumor marker in malignant melanoma [11].

Metastasis-associated lung adenocarcinoma transcript 1 (MALAT-1) lncRNA has been indicated in the metastatic potential of some cancers and involving in potential molecular mechanisms [12, 13]. Plasma long non-coding RNA MALAT1 is associated with distant metastasis in patients with epithelial ovarian cancer [14]. MALAT-1 model would prevent 30.2%-46.5% of unnecessary biopsies in PSA 4-10 ng/ml cohorts, without missing any high-grade cancers and demonstrated that urine MALAT-1 is a promising biomarker for predicting prostate cancer risk [15]. MALAT1 upregulation plays an important role in breast cancer development, and serum MALAT1 level may be a potential tumor marker for breast cancer diagnosis [16]. However, it is unclear whether plasma levels of MALAT1 may act as a biomarker for evaluating the development of metastasis in intracranial aneurysms (IA).

In the study, our results showed that the MALAT1 expression in intracranial aneurysm (IA) patients was significantly higher MALAT1 and was closely associations with hypertension history, rupture and Hunt-Hess level. Higher MALAT1 expression had short disease-free survival (DFS) and poor overall survival (OS) for patients. Multivariate Cox proportional analysis revealed that MALAT1 was an independent risk factor for predicting the IA patient prognosis. Thus, serum MALAT1 expression in IA patients may be a novel predicted biomarker for prognosis.

Materials and methods

Patients and clinical specimens

All of the intracranial aneurysms (IA) patients were collected at department of neurosurgery from July 2013 to Jun 2016. The patients with IA who had no ruptured aneurysms were normally diagnosed by digital subtraction angiography (DSA). Patients with ruptured aneurysms presented with SAH and were diagnosed by DSA. Healthy volunteers without a family history of SAH were recruited as the control group. The study was performed in according with the Declaration of Helsinki and was approved by the ethics committees of Department of Neurosurgery, Second Xiang-Ya Hospital of Central South University. Written informed consent was obtained from all participants or their first-degrees relatives for unconscious patients.

Sample processing

After informed consent, human peripheral blood samples were collected into EDTA-containing tubes at department of Neurosurgery, Second Xiang-Ya Hospital of Central South University. The samples were left to stand in coagulant tubes for 30 minutes at 4°C. The whole blood was centrifuged at 1800 g for 10 minutes and the supernatant was transferred into a fresh tube and was centrifuged again at 12000 g for 10 minutes. A total of 400 μ l serum was used for MALAT1 using a qRT-PCR analysis according to manufacturer's protocols.

Quantitative real-time PCR (qPCR)

The 400 μ L plasma was added with 600 μ L of Trizol Reagent (TAKALA, Dalian, China) and the total RNA was isolated according to the manufacturer's protocols. Reverse transcription was performed using the Universal cDNA Synthesis Master Mix kit (TAKALA, Dalian, China). QRT-PCR was performed using SYBR Green Master Mix (TAKALA, Dalian, China). The PCR reaction was 95°C for 15 s, followed by denaturation at 95°C for 5 s, annealing at 60°C for 34 s, and for a total of 40 cycles. The expression levels of MALAT1 were normalized to the internal control GAPDH. MALAT1, forward primer: CTTCCCTAGGGGATTTTCAGG, MALAT1, reverse primer: GCCCACAGGAACAAGTCCTA. GAPDH, forward primer: GTCAACGGATTTGGTCTGTATT and reverse primer: AGTCTTCTGGGTGGCAGTGAT. Real-time

Higher MALAT1 is a poor prognosis for intracranial aneurysm (IA)

Table 1. Association between MALAT1 expression and clinicopathological factors in intracranial aneurysm (IA) patients

Factors	Number of IA patients	MALAT1		p-value
		Low (n=46)	High (n=59)	
Sex				0.464
Female	46	22	24	
Male	59	24	35	
Age				0.206
≤60	62	24	38	
>60	43	22	21	
Aneurysm size (cm)				0.541
<10 cm	74	31	43	
>10 cm	31	15	16	
Smoking				0.833
Yes	49	22	27	
No	56	24	32	
Coronary heart disease history				0.376
Present	34	17	17	
No	71	29	42	
Hypertension history				0.006**
Yes	74	26	48	
No	31	20	11	
Blood glucose				0.224
>5 mmol/L	28	15	13	
<5 mmol/L	77	31	46	
Rupture				0.003**
Yes	69	23	46	
No	36	23	13	
Hunt-Hess level				0.002**
Level I-III	65	36	29	
Level IV-V	40	10	30	
Aneurysm location				0.666
Anterior circulation aneurysm	55	23	32	
Posterior circulation aneurysm	50	23	27	

**P<0.05.

PCR reactions were performed using the ABI-7500 system (Applied Biosystems, Carlsbad, CA, USA). The real-time PCRs were performed in triplicate. Relative expression fold change of mRNAs was calculated by the $2^{-\Delta\Delta Ct}$ method.

Statistical analysis

All of the statistical analysis was performed using SPSS.13.0 (SPSS Inc., Chicago, IL, USA). The Student's t-test was used for statistical comparisons of continuous and categorical variables as appropriate. The *p* values were

two-sided, and *P*<0.05 was considered to be statistically significant.

Results

MALAT1 is upregulated in serum of intracranial aneurysms (IA) patients

To investigate the interaction between intracranial aneurysms (IA) and MALAT1, RT-PCR analysis was performed to detect the expression level of MALAT1 in 105 cases intracranial aneurysms (IA) patients, and 40 normal controls. The results showed that serum MALAT1 was significantly highly expressed in intracranial aneurysms patients, compared with the normal groups (IAs vs controls: 1.45 ± 0.36 vs. 1.02 ± 0.21) (**Figure 1**, *P*<0.05). The positive expression was 68/98 and the mean expression level in the intracranial aneurysms group was 1.45 times as high as that in normal controls.

Correlation between MALAT1 expression and clinicopathologic factors in IA patients

To investigate the role of MALAT1 in intracranial aneurysms (IA) patients, we analyzed that the correlation between MALAT1 expression and clinicopathologic factors in IA patients. The

results showed that MALAT1 expression was significantly correlated with hypertension history, rupture and Hunt-Hess level in IA patients (**Table 1**, *P*<0.05). Thus, our results demonstrated that MALAT1 could play an important role in IA patients.

Correlation between MALAT1 expression and disease-free survival (DFS) an overall survival (OS) in IA patients

Furthermore, we detected the correlation between MALAT1 expression levels and disease-

Higher MALAT1 is a poor prognosis for intracranial aneurysm (IA)

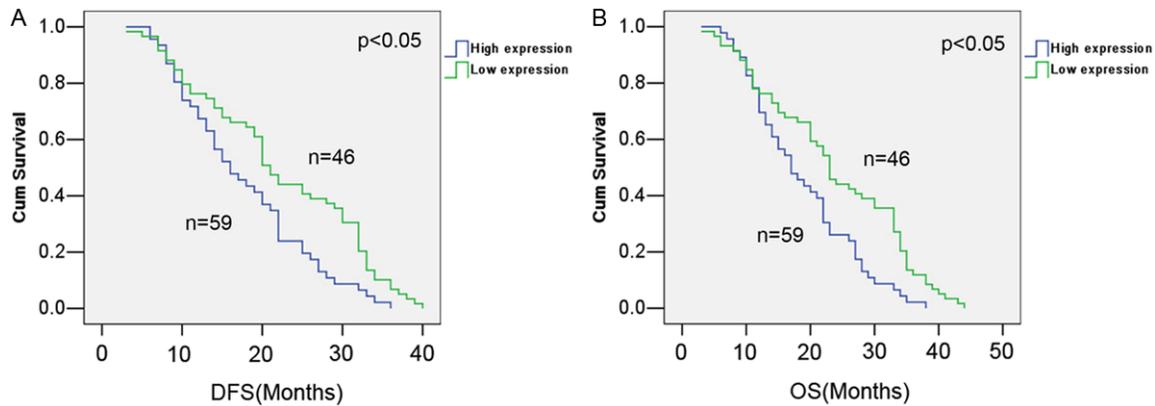


Figure 2. MALAT1 expression was significantly association with DFS and OS in IA patients. (A) Kaplan-Meier curve was performed to analyze the association between MALAT1 expression and DFS (B) Kaplan-Meier curve was performed to analyze the association between MALAT1 expression and OS in IA patients.

Table 2. Univariate Cox proportional hazards regression analysis for the risk factors of disease-free survival (DFS) for IA prognosis

Factors	HR	95% CI	p-value
Age	0.668	0.382-1.012	0.621
Sex	0.889	0.452-1.545	0.332
Aneurysm size (cm)	1.299	0.884-1.917	0.187
Smoking	1.066	0.552-1.709	0.248
Coronary heart disease history	1.223	0.654-2.033	0.089
Blood glucose	1.259	0.798-1.745	0.102
Aneurysm location	1.445	0.905-1.873	0.078
Hypertension	2.344	0.789-4.023	P<0.001
Rupture	2.997	0.989-5.133	P<0.001
Hunt-Hess grading	3.866	1.334-6.656	P<0.001
MALAT1 expression	2.879	1.228-4.798	P<0.001

Table 3. Multivariate Cox proportional hazards regression analysis for the risk factors of disease-free survival (DFS) for IA prognosis

Factors	HR	95% CI	p-value
Hypertension	1.956	0.556-3.789	P<0.001
Rupture	2.526	0.789-4.748	P<0.001
Hunt-Hess grading	3.338	1.056-5.895	P<0.001
MALAT1 expression	2.541	0.977-4.336	P<0.001

free survival (DFS) or the overall survival (OS) time in IA patients. The results showed that higher MALAT1 expression predicted a poor disease-free survival (DFS) or the overall survival (OS) in IA patients (**Figure 2A, 2B**, log-rank =9.43, P<0.05 or log-rank =8.92, P<0.05). Multivariate Cox proportional hazards regression analysis revealed that Hypertension, rup-

ture and Hunt-Hess grade and MALAT1 expression were risk factors to predict the disease-free survival (DFS) of IA patients and overall survival (OS) in IA patients (**Tables 2-5**, P<0.05). Thus, these results demonstrated serum MALAT1 expression acted as a predicted biomarker for IA patients.

Discussion

Previous studies have identified a number of risk factors result in the development of IAs, including sex, cigarette smoking, high blood pressure and so on. As a common vascular disease, rupture of intracranial aneurysm (IA) usually causes subarachnoid hemorrhage (SAH) [17, 18]. Circulating microRNAs had been reported to serve as novel biological markers for intracranial aneurysms. Wang *et al* reported that MicroRNA-29a was a potential biomarker in the development of intracranial aneurysms and patients with low miR-29a expression had longer disease-free survival (DFS) and overall survival (OS) than those with high miR-29a expression [19]. Wang *et al* found that development and prospective multicenter evaluation of the long noncoding RNA MALAT-1 was acted as a diagnostic urinary biomarker for prostate cancer [15]. In our study, our finding demonstrated that compared with healthy controls, the results showed that the MALAT1 expression in intracranial aneurysm (IA) patients was significantly higher MALAT1 expressions. Furthermore, we found that MALAT1 expression was closely

Higher MALAT1 is a poor prognosis for intracranial aneurysm (IA)

Table 4. Univariate Cox proportional hazards regression analysis for the risk factors of over survival (OS) for IA prognosis

Factors	HR	95% CI	p-value
Age	0.556	0.242-1.112	0.731
Sex	1.012	0.488-1.662	0.258
Aneurysm size (cm)	1.101	0.687-1.507	0.130
Smoking	0.911	0.617-1.346	0.294
Coronary heart disease history	1.044	0.322-1.868	0.152
Blood glucose	1.232	0.689-2.129	0.092
Aneurysm location	1.445	0.905-1.873	0.078
Hypertension	2.228	0.696-3.965	P<0.001
Rupture	2.774	0.956-4.989	P<0.001
Hunt-Hess grading	3.789	1.256-6.445	P<0.001
MALAT1 expression	2.795	1.165-4.669	P<0.001

Table 5. Multivariate Cox proportional hazards regression analysis for the risk factors of over survival (OS) for IA prognosis

Factors	HR	95% CI	p-value
Hypertension	1.884	0.446-3.422	P<0.001
Rupture	2.342	0.646-4.239	P<0.001
Hunt-Hess grading	2.838	0.924-4.955	P<0.001
MALAT1 expression	2.221	0.722-3.995	P<0.001

associations with hypertension history, rupture and Hunt-Hess level.

Aberrant Expression of microRNA-9 was found to contribute to development of intracranial aneurysm by suppressing proliferation and reducing contractility of smooth muscle cells [20]. Li *et al* showed that MiR-34b/c rs49-38723CC and TP53 Arg72-Pro polymorphisms was involved in the susceptibility to IA [21]. To investigate the role of MALAT1 in intracranial aneurysms (IA) patients, we analyzed the correlation between MALAT1 expression and disease-free survival (DFS) or overall survival (OS). The results showed that patients with higher MALAT1 expression had short disease-free survival (DFS) and poor overall survival (OS) than those with lower MALAT1 expression, indicating that serum MALAT1 expression levels might be useful biological markers for assessing the risk of IAs. Univariate and Multivariate Cox proportional hazards regression analysis revealed that MALAT1 expression, hypertension history, rupture and Hunt-Hess were independent risk factors for predicting the IA patient prognosis.

In summary, our study demonstrated that serum MALAT1 were significantly changed in patients with IAs. Our data suggest that plasma MALAT1 may be used as novel biological markers in assessing the likelihood of occurrence of IAs and also predicted a factor of IAs.

Acknowledgements

The study is supported by the National Natural Science Foundation of China (81571156).

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Yugang Jiang, Department of Neurosurgery, Second Xiang-Ya Hospital of Central South University, 139 Renmin Road, Changsha 410011, Hunan Province, People's Republic of China. Tel: 86-731-85295888; Fax: 86-731-85295888; E-mail: yugang666@21.com

References

- [1] Hussain S, Barbarite E, Chaudhry NS, Gupta K, Dellarole A, Peterson EC, Elhamady MS. Search for biomarkers of intracranial aneurysms: a systematic review. *World Neurosurg* 2015; 84: 1473-1483.
- [2] Barcelos GK, Tholance Y, Grousseau S, Renaud B, Perret-Liaudet A, Dailler F, Zimmer L. Outcome of poor-grade subarachnoid hemorrhage as determined by biomarkers of glucose cerebral metabolism. *Neurocrit Care* 2013; 18: 234-244.
- [3] Alg VS, Sofat R, Houlden H, Werring DJ. Genetic risk factors for intracranial aneurysms: a meta-analysis in more than 116,000 individuals. *Neurology* 2013; 80: 2154-2165.
- [4] Baker CJ, Fiore A, Connolly ES Jr, Baker KZ, Solomon RA. Serum elastase and alpha-1-antitrypsin levels in patients with ruptured and unruptured cerebral aneurysms. *Neurosurgery* 1995; 37: 56-61.
- [5] Low HL. Altered arterial homeostasis and cerebral aneurysms: a review of the literature and justification for a search of molecular biomarkers. *Neurosurgery* 2005; 56: E1166.
- [6] Liz J and Esteller M. lncRNAs and microRNAs with a role in cancer development. *Biochim Biophys Acta* 2016; 1859: 169-76.
- [7] Lu J, Xie F, Geng L, Shen W, Sui C, Yang J. Investigation of serum lncRNA-uc003wbd and lncRNA-AF085935 expression profile in patients

Higher MALAT1 is a poor prognosis for intracranial aneurysm (IA)

- with hepatocellular carcinoma and HBV. *Tumour Biol* 2015; 36: 3231-3236.
- [8] Tong YS, Wang XW, Zhou XL, Liu ZH, Yang TX, Shi WH, Xie HW, Lv J, Wu QQ, Cao XF. Identification of the long non-coding RNA POU3F3 in plasma as a novel biomarker for diagnosis of esophageal squamous cell carcinoma. *Mol Cancer* 2015; 14: 3.
- [9] Dong L, Qi P, Xu MD, Ni SJ, Huang D, Xu QH, Weng WW, Tan C, Sheng WQ, Zhou XY, Du X. Circulating CUDR, LSINCT-5 and PTENP1 long noncoding RNAs in sera distinguish patients with gastric cancer from healthy controls. *Int J Cancer* 2015; 137: 1128-1135.
- [10] Xu N, Chen F, Wang F, Lu X, Wang X, Lv M, Lu C. Clinical significance of high expression of circulating serum lncRNA RP11-445H22.4 in breast cancer patients: a Chinese population-based study. *Tumour Biol* 2016; 36: 7659-7665.
- [11] Ichigozaki Y, Fukushima S, Jinnin M, Miyashita A, Nakahara S, Tokuzumi A, Yamashita J, Kajihara I, Aoi J, Masuguchi S, Zhongzhi W, Ihn H. Serum long non-coding RNA, snoRNA host gene 5 level as a new tumor marker of malignant melanoma. *Exp Dermatol* 2016; 25: 67-9.
- [12] Hu ZY, Wang XY, Guo WB, Xie LY, Huang YQ, Liu YP, Xiao LW, Li SN, Zhu HF, Li ZG, Kan H. Long non-coding RNA MALAT1 increases AKAP-9 expression by promoting SRPK1-catalyzed SRSF1 phosphorylation in colorectal cancer cells. *Oncotarget* 2016; 7: 11733-11743.
- [13] Zhou X, Liu S, Cai G, Kong L, Zhang T, Ren Y, Wu Y, Mei M, Zhang L, Wang X. Long non coding RNA MALAT1 promotes tumor growth and metastasis by inducing epithelial-mesenchymal transition in oral squamous cell carcinoma. *Sci Rep* 2015; 5: 15972.
- [14] Chen Q, Su Y, He X, Zhao W, Wu C, Zhang W, Si X, Dong B, Zhao L, Gao Y, Yang X, Chen J, Lu J, Qiao X, Zhang Y. Plasma long non-coding RNA MALAT1 is associated with distant metastasis in patients with epithelial ovarian cancer. *Oncol Lett* 2016; 12: 1361-1366.
- [15] Wang F, Ren S, Chen R, Lu J, Shi X, Zhu Y, Zhang W, Jing T, Zhang C, Shen J, Xu C, Wang H, Wang H, Wang Y, Liu B, Li Y, Fang Z, Guo F, Qiao M, Wu C, Wei Q, Xu D, Shen D, Lu X, Gao X, Hou J, Sun Y. Development and prospective multicenter evaluation of the long noncoding RNA MALAT-1 as a diagnostic urinary biomarker for prostate cancer. *Oncotarget* 2014; 5: 11091-11102.
- [16] Miao Y, Fan R, Chen L and Qian H. Clinical significance of long non-coding RNA MALAT1 expression in tissue and serum of breast cancer. *Ann Clin Lab Sci* 2016; 46: 418-424.
- [17] Francis SE, Tu J, Qian Y, Avolio AP. A combination of genetic, molecular and haemodynamic risk factors contributes to the formation, enlargement and rupture of brain aneurysms. *J Clin Neurosci* 2013; 20: 912-918.
- [18] Tromp G, Weinsheimer S, Ronkainen A, Kuivaniemi H. Molecular basis and genetic predisposition to intracranial aneurysm. *Ann Med* 2014; 46: 597-606.
- [19] Wang WH, Wang YH, Zheng LL, Li XW, Hao F and Guo D. MicroRNA-29a: a potential biomarker in the development of intracranial aneurysm. *J Neurol Sci* 2016; 364: 84-89.
- [20] Luo J, Jin H, Jiang Y, Ge H, Wang J and Li Y. Aberrant expression of microRNA-9 contributes to development of intracranial aneurysm by suppressing proliferation and reducing contractility of smooth muscle cells. *Med Sci Monit* 2016; 22: 4247-4253.
- [21] Li L, Sima X, Bai P, Zhang L, Sun H, Liang W, Liu J, Zhang L, Gao L. Interactions of miR-34b/c and TP53 polymorphisms on the risk of intracranial aneurysm. *Clin Dev Immunol* 2012; 2012: 567586.