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Original Article

High expression of MTA3 predicts a poor prognosis for patients with colon cancer

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Abstract: Objectives: To elaborate the expression of MTA3 in colon cancer and correlation with prognosis as well as clinical index. Methods: We chose a colon cancer tissue microarray with follow-up information (containing 90 colon cancer specimens). Immunohistochemistry was applied to investigate the expression level of MTA3. The relationship between MTA3 expression and clinical index as well as the prognosis of colon cancer were statistically analyzed by SPSS software respectively. Results: MTA3 was specifically expressed in the nucleus of colon cancer tissue and para-carcinoma tissue, and there was a significant positive correlation between them (r=0.278, P=0.008). The clinical indexes correlation analysis showed that: the expression of MTA3 in colon cancer tissue was not correlated with clinical indexes (P>0.05); the expression of MTA3 in para-carcinoma tissue was significant positive correlated with M staging, clinical staging (r=0.264, P=0.013; r=0.222, P=0.039). Further survival analysis showed that the expression of MTA3 in colon cancer tissue was significant negative correlated with overall survival time of patients (35.7% VS 54.2%, P=0.030), and it was an independent predict factor (P=0.045); the expression of MTA3 in para-carcinoma tissue was not correlated with overall survival time (P=0.576). In addition, age, N staging and M staging were all independent predict factors of colon cancer patients, and they were significant negative correlated with prognosis (P<0.05). Conclusion: We hypothesized that: MTA3 was a clear oncogene in colon cancer. There may be multiple genes involve in the cancer promoting gene network of MTA3 in both cancer tissue and para-carcinoma tissue, they all increased the migration ability of cancer cell and reduced the survival time of patients. Next step, in order to further understand the molecular mechanism of MTA3 in colon cancer, we will carry out cytology research.

Keywords: MTA3, colon cancer, tissue microarray, immunohistochemistry, prognosis

Introduction

Colorectal cancer (CRC) is one of the third most mortal tumors around the world [1]. Although continual innovative therapy strategies had been investigated in the past two decades, there were still no effective methods to improve the prognosis of CRC patients. In order to identify novel targets for prediction and therapy as well as for the improvement of prognosis, researchers have to get a further insight into CRC.

The family of metastasis-associated protein (MTA) was found in the process of studying cancer metastasis in recent years, and they were an important part of the nucleosome remodeling and histone deacetylase (NuRD), can control the proteins activity by regulating the acetylation, as well as regulated several important signaling pathway by acetylation/deacetylation of the core proteins. They also participated in multiple biological processes involved in tumor cell invasion and metastasis [2, 3]. The MTA family consisted of three members: MTA1, MTA2 and MTA3. Among them, MTA1 and MTA2 were found to be related with the invasion and migration in a variety of cancer cells, including pancreatic cancer, esophageal cancer, colon cancer, and many other solid tumors, and also had significant negative correlation with the prognosis of the patients [4-7]. But there were very few researches about MTA3, and the conclusions were contradictory. Some studies showed MTA3 was an oncogene, for example, MTA3 had a cancer-promoting role

Table 1. Clinicopathologic factors of colon cancer patients

Clinical factor	Numbers (%)
All patients	90 (100%)
Gender	
Male	47 (52.2%)
Female	43 (47.8%)
Age	
≤65 years	34 (37.8%)
>65 years	54 (60.0%)
Lost	2 (2.2%)
Tumor size	
≤5 cm	46 (51.1%)
>5 cm	42 (46.7%)
Lost	2 (2.2%)
Pathological grade	
Grade I	5 (5.6%)
Grade II	49 (54.4%)
Grade III	36 (40.0%)
T stage	
T1	3 (3.3%)
T2	6 (6.7%)
T3	68 (75.6%)
T4	11 (12.2%)
Lost	2 (2.2%)
N stage	
N0	56 (62.2%)
N1	25 (27.8%)
N2	9 (10.0%)
M stage	
M0	87 (96.7%)
M1	2 (2.2%)
Lost	1 (1.1%)
cTNM	
Stage I	7 (7.8%)
Stage II	47 (52.2%)
Stage III	32 (35.6%)
Stage IV	2 (2.2%)
Lost	2 (2.2%)

in lung cancer: the expression of MTA3 in cancer tissue was significant higher than carcinoma adjacent tissue (P<0.01); the expression of MTA3 had a significant positive correlated with lymph node metastasis (P<0.05); the patients with high expression of MTA3 had a worse prognosis (P=0.000) [8, 9]. In addition, MTA3 was an independent predict factor of uterine non-endometriod carcinoma, and negative correlated with prognosis [10]. In another aspect, MTA3 showed tumor suppress function in breast cancer and gastroesophageal junction adenocarcinoma [11, 12]. For example, Hongmei Dong [12] found that: in the specimens of gastroesophageal junction adenocarcinoma, the mRNA and protein expression of MTA3 in cancer tissue was lower than para-carcinoma tissue; the patients with higher expression of MTA3 had better prognosis, and it was an independent predict factor (P≤0.001). Form the above results, we hypothesized that MTA3 may be involved in different gene regulatory networks, and played different biological functions. The correlation between MTA3 and CRC had not been reported.

In order to study the correlation between MTA3 and the occurrence, development, prognosis of CRC as well as to speculate the possible molecular mechanism, we chose a colon cancer tissue microarray with follow-up information (containing 90 colon cancer specimens), immunohistochemistry and statistical methods were applied to investigate the correlation between MTA3 and CRC, also speculated the biological function and molecular mechanism of MTA3.

Materials and methods

Colon cancer specimens

Colon cancer tissue microarray (HCol-Ade180-Sur-04) was obtained from Shanghai Outdo Biotech Co., Ltd, contained 90 carcinoma tissues and paired para-carcinoma tissues (1.5 cm from para-carcinoma tissue to cancer tissue).

The clinical data of patients were shown in **Table 1**; 47 cases were male, 43 cases were female; the age distribution from 24 to 90 years old; tumor size range from 2 cm to 15 cm. The clinical grading distribution: 7 cases were stage 1, 47 cases were stage 2, 32 cases were stage 3, 2 cases were stage 4, there were 2 cases missed clinical staging information.

The follow-up of HCC patients: The operation time was from July 2006 to May 2009 and the eventual follow-up time in September 2013, which followed 3.8-6.7 years. During this follow-up time, 49 patients were died of CRC, with a median follow-up time of 28 months (3-82 months); 34 patients were still alive, with a

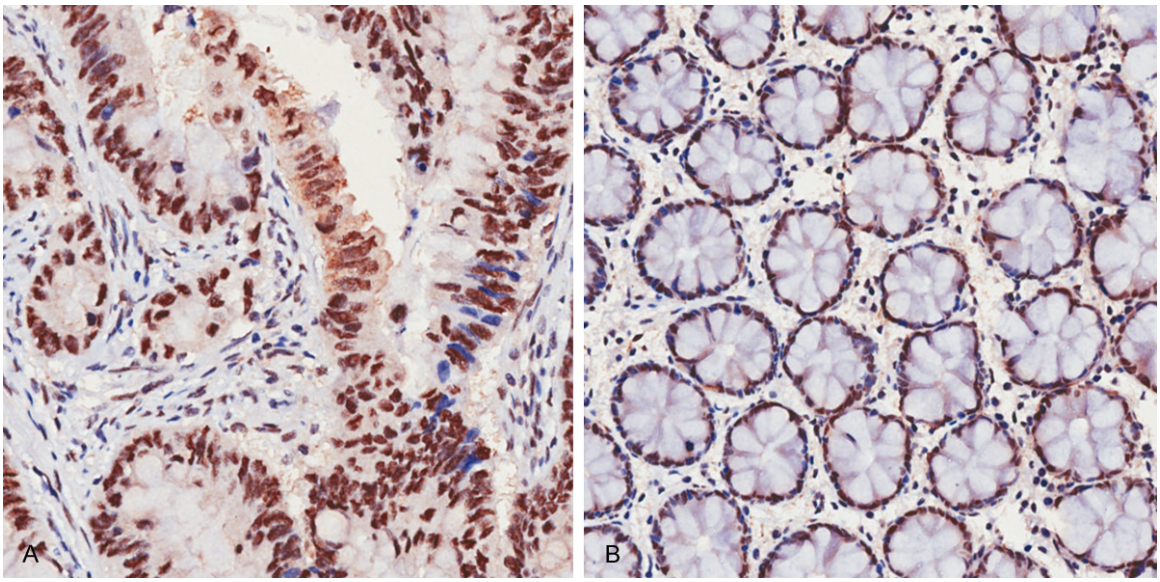

Figure 1. Immunohistochemistry results of MTA3 in colon cancer tissue and adjacent tissue. (A) MTA3 staining-positive rate in colon cancer tissue was slightly higher than (B) that in adjacent tissue, but the difference was not significant (90.17% ± 9.43% VS 88.99% ± 6.32%, P=0.318). Magnification: 200 ×.

median follow-up time of 88 months (87-97 months), there were 7 patients lost to follow-up in August 2012, and they were included in the survival group when statistical analysis. All patients were clinicopathologically diagnosed as colon cancer and received no extra treatment before surgery.

Immunohistochemistry

Two-step immunohistochemistry was used: antigen retrieval with EDTA in high temperature and high pressure. The Tissue sections were blocked with goat serum and subsequently incubated with primary antibody which anti-MTA3 (1:500, 14682-1-AP, Proteintech) at 4°C overnight. Then, it was incubated with secondary antibody (HRP-labeled anti-mouse antibody, DAKO). Washed with PBS, visualizing using diaminobenzidine (DAB) system and hematoxylin re-dying, observed and analyzed with microscope, randomly 3 high-magnification field were chosen under optical microscope and calculated more than 3 × 100 cells. The number of positive cells was calculated to account for the positive staining rate of the whole number of cells. Grouped with staining positive rate, when positive rate ≤90% were divided into low expression group, when positive rate >90% were divided into high expression group.

Statistical analysis

The positive rate of immunohistochemical staining was statistically analyzed. Associations between MTA3 expression in carcinoma tissues and para-carcinoma tissues were analyzed by paired T test. The correlation between MTA3 expression and clinicalpathological parameters of colon cancer were calculated by Spearman's correlation analysis. Univariate analysis between MTA3 and clinical data was evaluated using the Kaplan-Meier method and the log-rank test. Then, statistically significant variables in Univariate analysis would be included in COX multivariate regression survival analysis. $P<0.05$ was considered to be statistically significant.

Results

The expression pattern of MTA3 in colon cancer tissue and para-carcinoma tissues

The results of Immunohistochemistry indicated that MTA3 was localized in the nucleus in all CRC specimens. The specimens which positive rate >90% of MTA3 were divided into high expression group, there were 42 specimens with MTA3 high expression in the nuclear of cancer tissue, account for 46.67%; there were 23 specimens with MTA3 high expression in

Table 2. Study on differential expression of MTA3 in colon cancer tissue and adjacent tissue

MTA3 expression	Cases	Mean ± Std. Deviation	*P*-value
MTA3 expression in colon cancer tissue	89	90.17% ± 9.43%	0.318
MTA3 expression in para-carcinoma tissue	89	88.99% ± 6.32%	

Table 3. Study on the correlation between the expression of MTA3 in colon cancer tissue and adjacent tissue

			MTA3 expression (para-carcinoma tissue)
Spearman's rho	MTA3 expression (colon cancer tissue)	Correlation Coefficient	2.78
		Sig. (2-tailed)	0.08
		N	89

the nuclear of para-carcinoma tissue, account for 25.84%. Paired T-Test analysis indicated that: the positive rate of MTA3 in CRC tissue was higher than para-carcinoma tissue, but *P* value was not significant (90.17% ± 9.43% VS 88.99% ± 6.32%, P=0.318). The representative pictures of the immunohistochemistry were shown in **Figure 1**. The analysis was showed in **Table 2**.

Pearson correlation analysis was applied to study the correlation between the expression of MTA3 in colon cancer tissues and para-carcinoma tissues. The results indicated that: there was a significant positive correlation between the expression of MTA3 in colon cancer tissues and para-carcinoma tissues (r=0.278, P=0.008). Detailed analysis results were shown in **Table 3**.

The correlation between MTA3 expression and clinical index

Spearman's correlation analysis showed MTA3 expression in cancer tissue was not correlated with clinical indexes (P>0.05); MTA3 expression in para-carcinoma tissue was significant positive correlated with M staging and clinical staging (r=0.264, P=0.013; r=0.222, P=0.039), the patients with MTA3 high expression in para-carcinoma tissue had worse prognosis. The results were shown in **Table 4**.

The correlation between MTA3 expression, clinical indexes and prognosis of HCC

Kaplan-Meier analysis and log-rank test were applied to determine the association between MTA3 expression, clinical indexes and prognosis of CRC respectively. The results showed that: the expression of MTA3 in cancer tissues were negative correlated with overall survival time of CRC patients (35.7% vs 54.2%, P=0.030), the expression of MTA3 in para-carcinoma tissues were not correlated with prognosis of CRC patients (43.5% vs 47.0%, P=0.576). Detailed analysis results were shown in **Figure 2**. In addition, age, N staging, M staging and clinical staging were significant negative correlated with prognosis of patients (P=0.031, P=0.000, P=0.000, P=0.000), but the gender, tumor size, pathological grading and T staging were not correlated with prognosis (P=0.442, P=0.102, P=0.509, P=0.283).

COX multi-factors analysis indicated that MTA3 expression in cancer tissue was an independent predict factor (P=0.045); in addition, age, N staging and M staging were independent predict factors of colon cancer (P=0.002, P=0.000, P=0.004). The detailed analysis results were shown in **Table 5**.

Discussion

In order to relevance the correlation between MTA3 proteins and development, metastasis as well as prognosis of CRC, we carefully chose a colon cancer tissue microarray which contained 90 cases with follow-up information, IHC technique and statistical analysis were applied to study the clinical significance of MTA3 expression in the occurrence and development of CRC, and discussed the possible molecular mechanism.

The results of Immunohistochemistry indicated that MTA3 was localized in the nucleus in all

Table 4. Correlation analysis of MTA3 expression and clinical factor of colon cancer patients

Clinical factor	All cases (90)	MTA3 expression (colon cancer tissue)		Coefficient	*P*-Value	MTA3 expression (para-carcinoma tissue)		Coefficient	*P*-Value
		Low expression (48 cases)	High expression (42 cases)			Low expression (66 cases)	High expression (23 cases)		
Gender				0.086	0.419			0.059	0.583
Male	47	27	20			36	11		
Female	43	21	22			30	12		
Age				0.039	0.716			-0.161	0.137
≤65 years	34	19	15			22	12		
>65 years	54	28	26			42	11		
Lost	2	1	1			2	0		
Tumor size				-0.163	0.130			-0.096	0.376
≤5 cm	46	21	25			32	14		
>5 cm	42	26	16			32	9		
Lost	2	1	1			2	0		
Pathological grade				0.141	0.184			-0.024	0.824
Grade I	5	3	0			3	2		
Grade II	49	29	6			38	11		
Grade III	36	16	7			25	10		
T stage				0.140	0.193			0.019	0.864
T1	3	1	2			1	2		
T2	6	5	1			6	0		
T3	68	36	32			50	11		
T4	11	4	7			8	3		
Lost	2	2	0			1	7		
N stage				0.083	0.437			0.195	0.068
N0	56	32	24			45	11		
N1	25	11	14			16	8		
N2	9	5	4			5	4		
M stage				0.009	0.937			0.264	0.013
M0	87	46	41			66	20		
M1	2	1	1			0	2		
Lost	1	1	0			0	1		
cTNM				0.080	0.458			0.222	0.039
Stage I	7	4	3			6	1		
Stage II	47	26	21			38	9		
Stage III	32	15	17			21	10		
Stage IV	2	1	1			0	2		
Lost	2	2	0			1	1		

CRC specimens. The positive rate of MTA3 in CRC tissue was higher than para-carcinoma tissue, but *P* value was not significant (90.17% ± 9.43% vs 88.99% ± 6.32%, P=0.318). Spearman correlation analysis showed that: there was a significant positive correlation between the expression of MTA3 in colon cancer tissues and para-carcinoma tissues (r=0.278, P=0.008). These results indicated that MTA3 may be involved in the same gene regulatory network both in cancer tissue and para-carcinoma tissue and performed similar biological functions in colon cancer patients. The correlation analysis of clinical indexes showed that: the expression of MTA3 in colon cancer tissue was not correlated with clinical indexes (P>0.05); the expression of MTA3 in para-carcinoma tissue was positive correlated with M staging and clinical staging (r=0.264, P=0.013; r=0.222, P=0.039), the patients with MTA3 high expres-

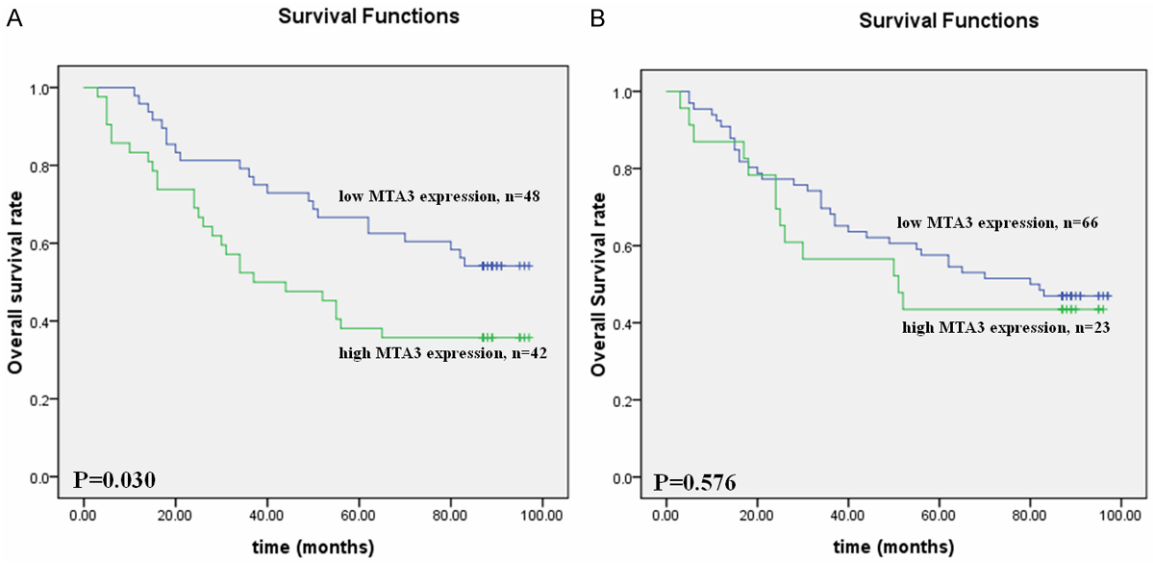

Figure 2. The analysis of correlation between MTA3 expression and prognosis of colon cancer patients. A: The correlation between the expression of MTA3 in colon cancer tissue and prognosis; the MTA3 expression in cancer tissue was significantly negatively correlated with the colon cancer patients' overall survival time (35.7% vs 54.2%, P=0.030). B: The correlation between the expression of MTA3 in para-carcinoma tissue and prognosis; while the MTA3 expression in para-carcinoma tissue was not correlated with the patients' prognosis (43.5% vs 47.0%, P=0.576).

Table 5. Analysis of independent prognostic factor in colon cancer patients by Cox Multivariate analysis variables

	B	SE	Wald	df	*P*-value	Exp (B)	95.0% CI for Exp (B)	
							Lower	Upper
MTA3 expression in colon cancer tissue	6.01	3.00	4.020	1	0.45	1.823	1.014	3.280
Age	1.091	3.55	9.437	1	0.02	2.976	1.484	5.967
N	1.553	3.88	16.021	1	0.00	4.725	2.209	10.107
M	2.669	9.28	8.281	1	0.04	14.428	2.343	88.866
cTNM stage	-4.65	3.68	1.600	1	2.06	6.28	306	1.291

sion in para-carcinoma tissue had worse prognosis. Further survival analysis was found the expression of MTA3 in cancer tissues were negative correlated with overall survival time of CRC patients (35.7% vs 54.2%, P=0.030), and it was an independent predict factor (P=0.045); the expression of MTA3 in para-carcinoma tissues were not correlated with prognosis of CRC patients (P=0.576). In addition, age, N staging and M staging were independent predict factors of colon cancer (P=0.002, P=0.000, P=0.004), and they were negative correlated with prognosis (P<0.05). In conclusion, firstly, MTA3 was a clear oncogene in colon cancer. Secondly, there may multiple genes involve in the cancer promoting gene network of MTA3 in both cancer tissue and para-carcinoma tissue, they increased the migration ability of cancer cell and reduced the survival time of patients.

The molecular mechanism of MTA3 in tumor suppression was studied in breast cancer, esophageal cancer and so on. Fujita [11] found that: loss expression of estrogen receptor or MTA3 can upregulate Snail expression and downregulate E-cadherin expression, then leaded to epithelial mesenchymal transition of tumor cells and promoted the invasive growth of tumor cells. The results of this study clearly indicated that MTA3 is a tumor suppressor in breast cancer. Hongmei Dong [12] found that: there were the same gene regulatory pathway of MTA3/Snail/E-cadherin in gastric esophageal junction cancer, and MTA3 was one of the

leading factors. The molecular mechanism of carcinogenesis in MTA3 was reported on the cytological study of lung cancer. Haiying Li found that: downregulated MTA3 expression with siRNA in A549 and H157 cell lines can reduce the expression of cyclinA, cyclinD1 and p-Rb, also can arrest the cells in G1, then inhibited the cell growth [8]. Meanwhile, they also found downregulated MTA3 can promote apoptosis of lung cancer cell lines, and upregulated the expression of Bax, Cleved-Caspase3, p-PARP, downregulated Bcl-2 [13]. Another study showed that: mir-495 could suppress the proliferation and migration ability in A549 and Calu-3 cell lines, the target gene of mir-495 was MTA3 [14]. Combined our results, we hypothesized that: in colon cancer, MTA3 also promoted the proliferation and migration of tumor cells through a similar gene regulation pathway in lung cancer, in this process, there may not only include the genes in colon cancer tissue, but also include the genes in para-carcinoma tissue, these genes were involved in the regulation network of MTA3, and finally affected the prognosis of patients.

In conclusion, the clinical relevance of MTA3 and colon cancer was studied in our experiment, and found MTA3 was an oncogene in CRC for the first time. Next step, in order to further understand the molecular mechanism of MTA3 in the occurrence and development of CRC, we planned to knockdown and overexpression MTA3 in multiple CRC cell lines.

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Disclosure of conflict of interest

None.

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References

[1] Cunningham D, Atkin W, Lenz HJ, Lynch HT, Minsky B, Nordlinger B, Starling N. Colorectal cancer. Lancet 2010; 375: 1030-1047.

[2] Bowen NJ, Fujita N, Kajita M, Wade PA. Mi-2/NuRD: multiple complexes for many purposes. Biochim Biophys Acta 2003; 1677: 52-57.

[3] Lai AY, Wade PA. Cancer biology and NuRD: a multifaceted chromatin remodelling complex. Nat Rev Cancer 2011; 11: 588-596.

[4] Qian H, Lu N, Xue L, Liang X, Zhang X, Fu M, Xie Y, Zhan Q, Liu Z, Lin C. Reduced MTA1 expression by RNAi inhibits in vitro invasion and migration of esophageal squamous cell carcinoma cell line. Clin Exp Metastasis 2005; 22: 653-62.

[5] Liu YP, Shan BE, Wang XL, Ma L. Correlation between MTA2 overexpression and tumour progression in esophageal squamous cell carcinoma. Exp Ther Med 2012; 3: 745-749.

[6] Y Y, Feng ST, Xiao MM, Li Y, Yang L, Gong J. MTA1 promotes proliferation and invasion in human gastric cancer cells. Onco Targets Ther 2015; 8: 1785-94.

[7] Sabatier R, Finetti P, Cervera N, Lambaudie E, Esterni B, Mamessier E, Tallet A, Chabannon C, Extra JM, Jacquemier J, Viens P, Birnbaum D, Bertucci F. A gene expression signature identifies two prognostic subgroups of basal breast cancer. Breast Cancer Res Treat 2011; 126: 407-20.

[8] Li H, Sun L, Xu Y, Li Z, Luo W, Tang Z, Qiu X, Wang E. Overexpression of MTA3 Correlates with Tumor Progression in Non-Small Cell Lung Cancer. PLoS One 2013; 8: e66679.

[9] Zheng SG, Du YW, Chu HY, Chen XD, Li P, Wang YY, Ma YY, Wang HQ, Zang WQ, Zhang GJ, Zhao GQ. Analysis of MAT3 gene expression in NSCLC. Diagn Pathol 2013; 8: 1-7.

[10] Mylonas I, Bruning A. The metastasis-associated gene MTA3 is an independent prognostic parameter in uterine non-endometrioid carcinomas. Histopathology 2012; 60: 665-670.

[11] Fujita N, Jaye DL, Kajita M, Geigerman C, Moreno CS, Wade PA. MTA3, a Mi-2/NuRD complex subunit, regulates an invasive growth pathway in breast cancer. Cell 2003; 113: 207-219.

[12] Dong HM, Guo H, Xie LX, Wang G, Zhong XY, Khoury T, Tan DF, Zhang H. The Metastasis-Associated Gene MTA3, a Component of the Mi-2/NuRD Transcriptional Repression Complex, Predicts Prognosis of Gastroesophageal Junction Adenocarcinoma. PLoS One 2013; 8: e62986.

[13] Li HY, Wang QL, Zhang L, Bao HJ, Zhang H. Regulation Mechanism of MTA3 in the Apoptosis of NSCLC Cells. Zhongguo Fei Ai Za Zhi 2015; 18: 610-5.

[14] Chu H, Chen X, Wang H, Du Y, Wang Y, Zang W, Li P, Li J, Chang J, Zhao G, Zhang G. MiR-495 regulates proliferation and migration in NSCLC by targeting MTA3. Tumour Biol 2014; 35: 3487-94.