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Original Article

Correlation of metastasis-associated protein expression with prognosis and chemotherapy in nasopharyngeal carcinoma

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Abstract: The aim of this study was to elaborate the correlation between metastasis-associated protein (MTA) family and the occurrence, progression, prognosis and chemotherapy efficiency in nasopharyngeal carcinoma (NPC). The expression of MTA1, MTA2 and MTA3 protein were detected by immunohistochemistry in a tissue microarray (TMAs) which contains tissue samples of 152 NPC patients embedded by formalin-fixed paraffin. The MTA proteins were mainly expressed in the nuclei of NPC tissues and the correlations between MTAs expression and clinical parameters as well as prognosis of NPC patients showed ethnical differences according to statistically analysis. The results showed that in Han ethnic group, MTA1 expression was positively correlated with N staging, while the expression of MTA2 was negatively correlated with age, and the expression of MTA3 was positively correlated with gender. Patients with high MTA1 expression had poorprognosis. In Zhuang ethnic group, only MTA3 expression was positively correlated with age, recurrence and metastasis of NPC patients; neither MTA1 nor MTA2 expression had any correlation with clinical indexes. Patients with high MTA3 expression had unfavorable prognosis. In addition, our results showed that overall survival among Zhuang NPC patients with low expression of MTA2 increased significantly owing to "carboplatin + fluorouracil" chemotherapy. This therapeutic success, however, did not translate to longer overall survival among Han NPC patients. The biological function of MTA protein family in NPC patients was different among different ethnic groups. In conclusion, we demonstrated that MTAs had a certain tumor promoting function in patients with NPC, and the biological functions of MTAs might be ethnic differences, which suggesting MTAs to be important markers for guiding clinical treatment of NPC.

Keywords: MTAs, nasopharyngeal carcinoma (NPC), tissue microarray, immunohistochemistry, survival time, chemotherapy

Introduction

Nasopharyngeal carcinoma (NPC) is the category of the head and neck cancer, the incidence of which is as low as 0.5/100000 people in western countries. However, in Asia, especially in southern China, the incidence of NPC is up to 30/100000 people [1]. Although radiotherapy and chemotherapy can improve the prognosis of patients [2, 3], the patients' 5-year survival rate remains low due to the high rate of recurrence or distant metastasis [4]. Therefore, study on the molecular markers associated with recurrence, distant metastasis or individualized treatment, might improve the clinical therapeutic effect and reduce mortality for this disease.

The family of metastasis-associated proteins (MTA), consists of 3 members, MTA1, MTA2 and MTA3, play a key role in cancer metastasis, nucleosome remodeling, acetylation of protein- and several important signaling pathways by acetylation/deacetylation of the core proteins [5, 6]. MTA1 and MTA2 were related to invasion and migration of many cancer cells, including

Table 1. Clinical data of NPC patients in various ethnic groups

Clinical data	Han Ethnic (56 cases)	Zhuang Ethnic (91 cases)	Yao Ethnic (3 cases)	Dong Ethnic (1 cases)	Melao Ethnic (1 cases)	Total (152 cases)
Gender						
Male	39	70	1	1	1	112
Female	17	21	2	0	0	40
Age						
≤50 years	28	55	2	1	0	86
>50 years	28	36	1	0	1	66
T staging						
T1-T2	27	46	1	0	0	74
T3-T4	29	45	2	1	1	78
N staging						
N0	21	25	0	0	1	47
N1-3	35	66	3	1	0	105
M staging						
M0	55	87	3	1	1	147
M1	1	4	0	0	0	5
Clinical staging						
Stage I	2	3	0	0	0	5
Stage II	21	35	1	0	0	57
Stage III	17	26	0	1	1	45
Stage IV	16	27	2	0	0	45
Recurrence and metastasis						
No	45	72	2	0	1	120
Yes	11	19	1	1	0	32
Radiotherapy						
No	0	0	0	0	0	0
Yes	56	91	3	1	1	152
Chemotherapy						
No	17	28	0	1	1	57
Yes	39	53	3	0	0	95
Follow up						
Alive	29	48	0	0	0	77
Died	26	41	2	1	1	71
Lost	1	2	1	0	0	4

pancreatic cancer, esophageal cancer, colon cancer, and many other solid tumors, and also had significant negative correlation with prognosis [7-12]. Study on the correlation between MTA3 and cancer and reveal its contradict role in different cancer has important implications for NPC treatment and diagnosis [13-15].

There were only a few articles revealed the relationship between the MTA family and NPC, and most of the studies were focused on MTA1. For example, Li et al found that MTA1 expression was positively correlated with N stage, clinical stage and distant metastasis of NPC (P=0.02, P=0.04, P<0.01), and the OS and the distant metastasis-free survival (DMFS) were significantly worse in the patients with high MTA1 expression (P<0.01). The results of Li et al suggest that nuclear overexpression of MTA1 correlates significantly with worse DMFS (P=0.02) and poorer OS (P=0.03) in NPC. In addition, Song et al found that MTA1 promotes NPC cell proliferation via enhancing G1 to S phase transition, leading to increased tumor growth. However, there was hardly any report revealed the correlation between MTA2, MTA3 and NPC [16].

In this paper, we elaborated the correlation between the expression of MTA1/MTA2/MTA3 and the occurrence, progression and prognosis of NPC based on the method of immunohistochemistry, in combination with tissue microarray technique, supporting the clinical individualized treatment of NPC.

Materials and methods

NPC specimens

The samples of NPC (152 cases) and verbal informed consent from patients in this study were obtained from the People's Hospital of Guangxi Zhuang Autonomous Region. This retrospective study using the surgical resection specimens from the year 2006 to 2008, when the patient's informed consent could not obtained at that time. Nevertheless, we consulted the patient's opinion at the time of follow-up and obtained the verbal informed consent, recorded on the original medical record card of our department and reported to the Ethics Committee of the People's Hospital of Guangxi Zhuang Autonomous Region.

Clinical data of NPC patients: 152 cases of patients were all non-keratin NPC, 2 cases of which is type II NPC of the World Health Organization, and 150 cases of World Health Organization type III NPC. 112 male cases and 40 female cases in detailed. The age distribution was range from 18 to 77 years old, and the distribution of clinical stages were 5 cases of stage I, 57 cases of stage II, 45 cases of stage III, and 45 cases of stage IV. The distribution of ethnicity were 56 cases of Han, 91 cases of Zhuang, 3 cases of Yao, 1 case of Dong and 1 case of Melao. The detailed clinical data were shown in **Table 1**. The follow-up information of NPC patients was shown as follows: the first diagnostic time was from June 2006 to October 2008, and the final time of follow-up was January 2014, 63-91 months in total. During this follow-up time, there were 32 patients with recurrence and metastasis, while there were 71 patients died of NPC, with a median follow-up time of 24 months (5 months-71 months); 77 patients were still alive, with a median follow-up time of 72 months (63 months-88 months); another 4 patients were lost to follow-up, not included in the survival analysis. The patients' 5-year overall survival rate was 52.03%, while the disease-free survival rate of 5 years was 51.01%. All patients were clinic pathologically diagnosed as non-keratin NPC.

The treatment for NPC patients: all 152 patients were treated with radiotherapy, 95 patients of whom received chemotherapy (carboplatin + fluorouracil). The details of radiotherapy: 8 MVX or 60 Co-gamma line were used to irradiate face and neck field, and the total dose of the primary lesion was 65-75 GY/6.5-7.5 W, while the dose of neck was 50-60 GY. The chemotherapeutic regimen for the treatment of NPC patients were shown as follows: patients received intravenous infusion of 200 mg/m^2 carboplatin at the first day; and from day 2 to day 5, patients received fluorouracil with a dose of 500 mg/m^2 through micro pump. Taking 28 days as 1 cycle, patients received 2 cycles of chemotherapy.

Tissue microarray

The NPC tissue microarray was made by Shanghai Out do Biotech Co., Ltd. All donor paraffin-embedded sections were resected and stained by hematoxylin-eosin (HE). The typical pathological sites on HE slices was labeled by pathologists, then be drilled on the blank recipient paraffin (diameter was 1.5 mm) by using tissue microarray instrument (Beecher Instruments. Inc.). Subsequently, 4-5 μm slices from tissue section were took by slicer (Leica, Germany) continuously, and then be attached to anti-off microslides.

Immunohistochemistry

Two-step immunohistochemistry assay: sections were treated with EDTA buffer under high pressure at high temperature to retrieval antigen, blocked with goat serum. Subsequently, sections were incubated with primary antibody which anti-MTA1 (1:2000, ab84136, Abcam)/ anti-MTA2 (1:3000, sc-55566, Santa Cruz)/ anti-MTA3 (1:1000, 14682-1-AP, Proteintech) at 4°C overnight. Then it was incubated with secondary antibody (HRP-labeled anti-mouse antibody, DAKO), washed with PBS, visualized 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. Scored and grouped with positive staining rate and intensity, the positive staining rate was defined

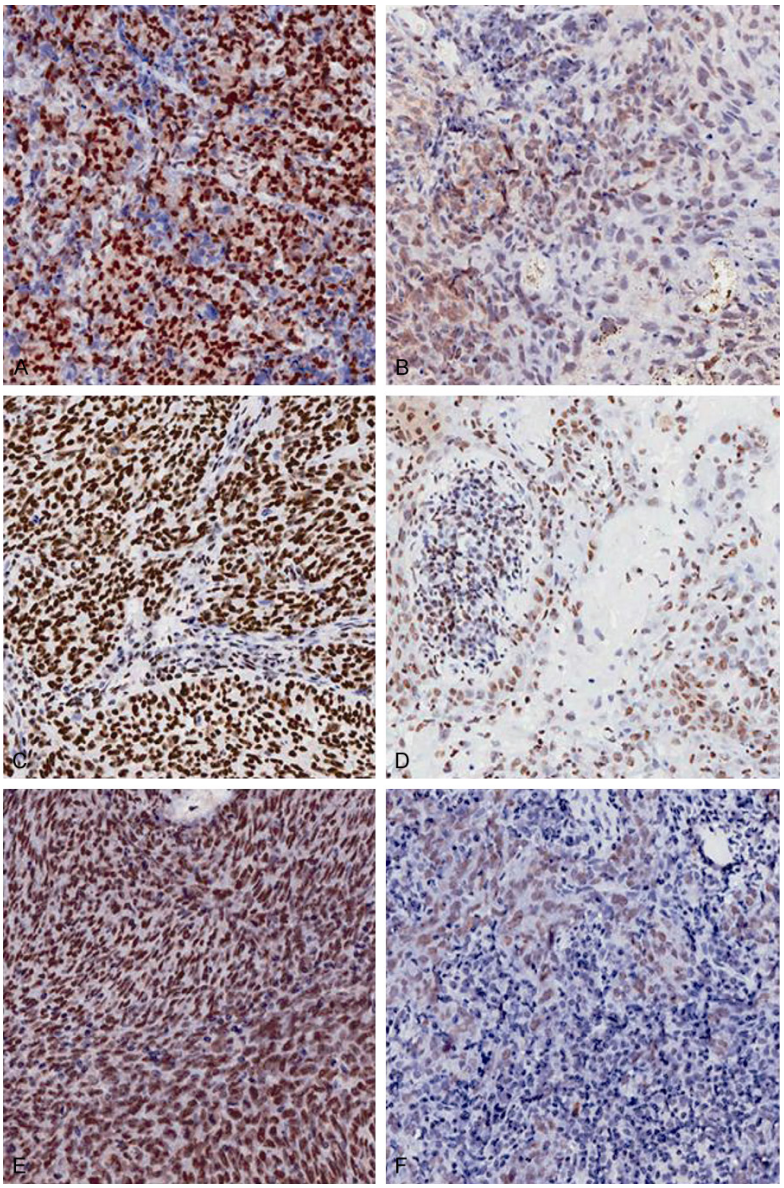

Figure 1. Representative immunohistochemistry of MTA in different stage NPC tissues and non-NPC tissues. A: MTA1 high expression; B: MTA1 low expression; C: MTA2 high expression; D: MTA2 low expression; E: MTA3 high expression; F: MTA3 low expression (Magnification times: ×200).

according to the proportion of positively stained cancer cells: "Negative" is 0, "≤20%" for 1, "21%-40%" for 2, "41-60%" for 3, "61-80%" for 4, "81-100%" for 5. The score of staining intensity: "Negative" is 0, "1+" for 1, "2+" for 2, "3+" for 3. Patients were divided into two groups according to the scores "positive staining rate score" multiply "staining intensity score". Equal or less than 8 were divided into low expression group, and more than 8 were divided into high expression group. Negative control was carried out by adding PBS instead of primary antibody.

Statistical analysis

Data was statistically analyzed using SPSS software version 17.0 in both Han and Zhuang ethnic groups. Spearman rank correlation coefficient was used to calculate the relationship between the MTAs expression and the clinical factors. Survival analysis between MTA expression, clinicaldata, chemotherapy and the overall survival time was evaluated by Kaplan-Meier method and the log-rank test, and 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

Different correlations among MTA1, MTA2 and MTA3 expression in Han and Zhuang ethnicities

The results of immunohistochemistry indicated that MTA1, MTA2 and MTA3 were all mainly localized at the nucleus of NPC tissues in both Han and Zhuang ethnicities. The representative pictures of the immunohistochemistry were shown in **Figure 1**.

Ruling out the off-point experimental cases,the IHC data of MTA was shown as follows: in Han group, 44 cases of MTA1, 56 cases of MTA2, 47 cases of MTA3; and in Zhuang ethnic group, 77 cases of MTA1, 91 cases of MTA2, 78 cases of MTA3 were calculated in this statistical analysis. The other 5 cases (3 cases of Yao, 1 case of Dong, 1 case of Melao) were not included in the statistical analysis. Spearman's correlation analysis showed that: in Han ethnic group, MTA1 and MTA2 expression had a positive cor-

Table 2. Correlation among MTA1, MTA2 and MTA3 expression in Han and Zhuang NPC patients

Ethnic			MTA1 expression	MTA2 expression	MTA3 expression
Han	MTA1 expression	Spearman's Correlation Coefficient	1.000	0.320*	0.278
		P value		0.034	0.071
		Number	44	44	43
	MTA2 expression	Spearman's Correlation Coefficient	0.320*	1.000	0.344*
		P value	0.034		0.018
		Number	44	56	47
Zhuang	MTA1 expression	Spearman's Correlation Coefficient	1.000	0.410**	0.563**
		P value		0.000	0.000
		Number	77	77	77
	MTA2 expression	Spearman's Correlation Coefficient	0.410**	1.000	0.189
		P value	0.000		0.097
		Number	77	91	78

**Correlation is significant at the 0.01 level (2-tailed). *Correlation is significant at the 0.05 level (2-tailed).

Table 3A. Correlation between clinical data and MTA expression in Han NPC patients

Clinical data	MTA1 expression		*P* value	MTA2 expression		*P* value	MTA3 expression		*P* value
	High (35)	Low (9)		High (7)	Low (49)		High (6)	Low (41)	
Gender			0.656			0.914			0.035
Male	26	6		5	34		2	31	
Female	9	3		2	15		4	10	
Age			0.064			0.044			0.094
≤50 years	15	7		6	22		1	19	
>50 years	20	2		1	27		5	22	
T staging			0.090			0.195			0.307
T1-T2	19	2		5	22		4	18	
T3-T4	16	7		2	27		2	23	
N staging			0.011			0.760			0.538
N0	11	7		3	18		3	15	
N1-3	24	2		4	31		3	26	
M staging			0.618			0.709			0.706
M0	34	9		7	48		6	40	
M1	1	0		0	1		0	1	
Clinical staging			0.740			0.633			0.251
Stage I	1	1		0	2		0	2	
Stage II	14	1		4	17		4	12	
Stage III	10	5		2	16		1	15	
Stage IV	10	2		1	14		1	12	
Recurrence/metastasis			0.118			0.709			0.210
No	27	9		6	39		6	32	
Yes	8	0		1	10		0	9	

relation (r=0.320, P=0.034), and so does the correlation between the MTA2 and MTA3 expression (r=0.344, P=0.018), while the expression of MTA1 was not correlated with MTA3 expression (P=0.071). In Zhuang ethnic group, the expression of MTA1 was positive correlated with MTA2 expression (r=0.410, P=0.000), and the expression of MTA1 was positive correlated

Table 3B. Correlation analysis of clinical data and MTA expression in Zhuang NPC patients

Clinical data	MTA1 expression		*P* value	MTA2 expression		*P* value	MTA3 expression		*P* value
	High (67)	Low (10)		High (19)	Low (72)		High (18)	Low (60)	
Gender			0.284			0.112			0.326
Male	51	6		12	58		15	43	
Female	16	4		7	14		3	17	
Age			0.791			0.065			0.007
≤50 years	44	7		15	40		7	44	
>50 years	23	3		4	32		11	16	
T staging			0.102			0.477			0.567
T1-T2	35	8		11	35		11	32	
T3-T4	32	2		8	37		7	28	
N staging			0.471			0.486			0.688
N0	21	8		4	21		6	17	
N1-3	46	2		15	51		12	43	
M staging			0.291			0.838			0.672
M0	65	9		18	69		17	58	
M1	2	1		1	3		1	2	
Clinical staging			0.120			0.701			0.367
Stage I	2	0		0	3		1	1	
Stage II	26	7		9	26		6	27	
Stage III	20	2		5	21		4	19	
Stage IV	19	1		5	22		7	13	
Recurrence/metastasis			0.949			0.984			0.028
No	53	8		15	57		11	51	
Yes	14	2		4	15		7	9	

with MTA3 expression (r=0.563, P=0.000), but the expression of MTA2 was not correlated with MTA3 expression (P=0.097). The results were shown in **Table 2**.

The correlation between MTAs expression and clinical index

Spearman's correlation analysis was used to investigate the correlation between MTAs expression and clinical parameters of NPC patients in both two ethnic groups. The results showed that: in Han ethnic group, the expression of MTA1 was correlated with lymph node (N) staging (r=0.380, P=0.011) positively, so does the expression of MTA3 with gender (r=0.308, P=0.035), whereas the expression of MTA2 was negatively correlated with age (r=-0.270, P=0.044). In Zhuang ethnic group, MTA3 expression was positively correlated with age, recurrence/metastasis of NPC patients (r=0.305, P=0.007; r=0.249, P=0.028), while neither the expression of MTA1 nor MTA2 had any significant correlation with the clinical indexes (P>0.05). Theresults were shown in **Table 3A** and **3B**.

The correlation between MTA expression, clinical index and the prognosis of NPC patients

In Han ethnic group, the patients with high MTA1 expression had worse prognosis compared to those with low MTA1 expression (40.0% VS 88.9%, P=0.026) (**Figure 2A**). However, neither MTA2 expression nor MTA3 expression was closely related to survival time (P>0.05) (**Figure 2C** and **2E**). Meanwhile, gender, age, M staging, recurrence and metastasis were all correlated with the prognosis of patients significantly (P=0.029, P=0.021, P=0.045, P=0.000). In Zhuang ethnic group, however, neither MTA1 expression nor MTA2 expression was correlated with the overall survivaltime significantly (P>0.05) (**Figure 2B** and **2D**), while the patients with high expression of MTA3 had worse prognosis (38.9% VS 62.1%, P=0.031) (**Figure 2F**). The N staging, M staging, clinical staging as well as recurrence and

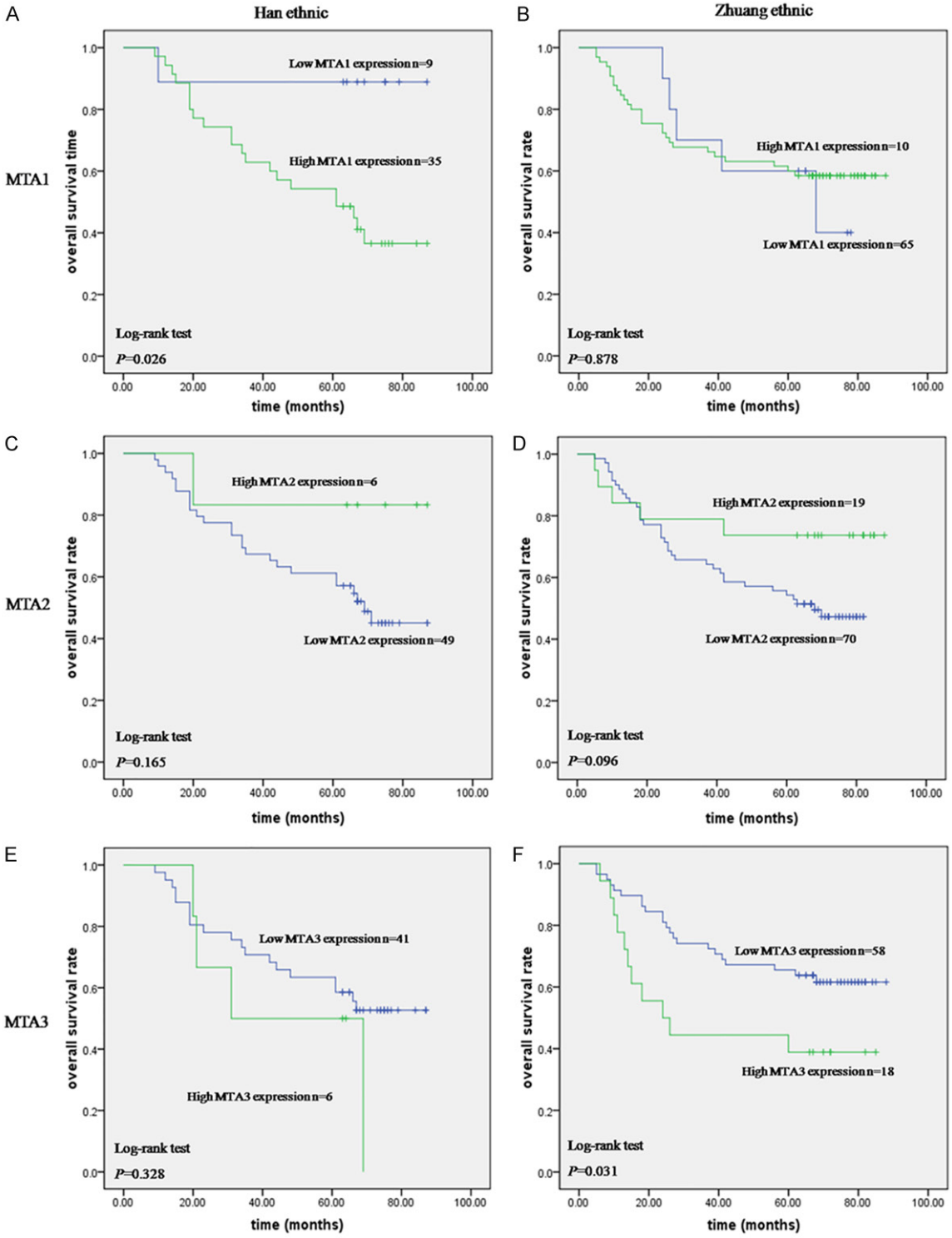

Figure 2. The correlation between MTA expression and prognosis of NPC. A: The relationship between MTA1 expression and OS time of Han NPC patients; B: The relationship between MTA1 expression and OS time of Zhuang NPC patients; C: The relationship between MTA2 expression and OS time of Han NPC patients; D: The relationship between MTA2 expression and OS time of Zhuang NPC patients; E: The relationship between MTA3 expression and OS time of Han NPC patients; F: The relationship between MTA3 expression and OS time of Zhuang NPC patients.

Table 4A. COX multivariate regression analysis of the independent predictors of OS in Han NPC patients

	P-value	Exp (B)	95.0% CI for Exp (B)	
			Lower	Upper
MTA1 expression	0.254	3.424	0.413	28.385
Gender	0.424	0.572	0.145	2.250
Age	0.107	2.593	0.814	8.265
M staging	0.252	4.061	0.370	44.622
Recurrence or metastasis	0.029	2.956	1.115	7.839

Table 4B. COX multivariate regression analysis of the independent predictors of OS in Zhuang NPC patients

	P-value	Exp (B)	95.0% CI for Exp (B)	
			Lower	Upper
MTA3 expression	0.294	1.547	0.685	3.490
Gender	0.277	0.545	0.182	1.629
T staging	0.211	0.433	0.116	1.609
N staging	0.029	3.035	1.120	8.226
M staging	0.031	5.387	1.165	24.904
Clinical staging	0.011	2.876	1.277	6.478
Recurrence or metastasis	0.012	2.869	1.259	6.538

metastasis were all significantly correlated with the prognosis of patients (P=0.019, P=0.001, P=0.036, P=0.000, P=0.000, P=0.000). Finally, a COX multivariate regression was performed including all statistically significant variables. The results indicated that: in Han ethnic group, MTA1 expression was not an independent predict factor (P=0.254), but only recurrence and metastasis was an independent predict factor (P=0.029); in Zhuang ethnic group, MTA3 expression was not an independent predict factor (P=0.294), but the N staging, M staging, clinical staging as well as recurrence and metastasis were all independent predict factors of the group patients (P=0.029, P=0.031, P=0.011, P=0.012). The results were shown in **Table 4A** and **4B**.

Different chemotherapy efficiency on NPC patients with different ethnics/MTAs expression

The proportions of NPC patients received chemotherapy in this study were different in ethnicities. 39 out of 56 (69.64%) Han NPC patients and 53 out of 91 (58.24%) Zhuang NPC patients were received chemotherapy. The detailed information could be found in **Table 1**. Survival analysis indicated that Han NPC patients received chemotherapy did not prolongoverall survival time in this studyobviously (52.6% VS 52.9%, P=0.859) (**Figure 3A**). However, the Zhuang NPC patients received chemotherapy survive longer time than those did not, although the correlation was not significantly (61.5% VS 43.2%, P=0.077) (**Figure 3B**).

Subsequently, we divided the Zhuang NPC patients into several subgroups according to MTAs expression level, and analyzed the correlation between the chemotherapy and the prognosis respectively. The results showed that: firstly, in the subgroup of MTA1 high expression (65 cases), patients treated with chemotherapy had better prognosis, even though the *P* value was >0.05 (65.8% VS 48.1%, P=0.133); however, in the MTA1 low expression subgroup (10 cases), the prognosis of patients with chemotherapy was worse, but not significant (28.6% VS 100.0%, P=0.096) (**Figure 4A** and **4B**). Secondly, in the MTA2 high expression subgroup (19 cases), the prognosis of patients with chemotherapy was worse, but the association was not significant (61.5% VS 100.0%, P=0.096); but in the low expression of MTA2 group (70 cases), the prognosis of patients with chemotherapy was significantly better (61.5% VS 32.3%, P=0.009) (**Figure 4C** and **4D**). At last, the prognosis was not affected by chemotherapy in neither MTA3 high expression group (18 cases) nor MTA3 low expression group (58 cases) (P=0.861, P=0.922) (**Figure 4E** and **4F**).

Discussions

In order to relevant the correlation between MTA proteins, treatment of NPC patients and prognosis, we collected 152 cases of NPC which diagnosed between June 2006 and October 2008, with clinical pathological data, therapeutic regimen and the follow-up information. Immunohistochemical technique and statistical analysis were applied to study the correlation between the expressions of MTA1, MTA2, MTA3 and the clinical significance in Han and Zhuang ethnic groups respectively. The NPC tissue microarray was made by Shanghai Out do Biotech Co., Ltd.

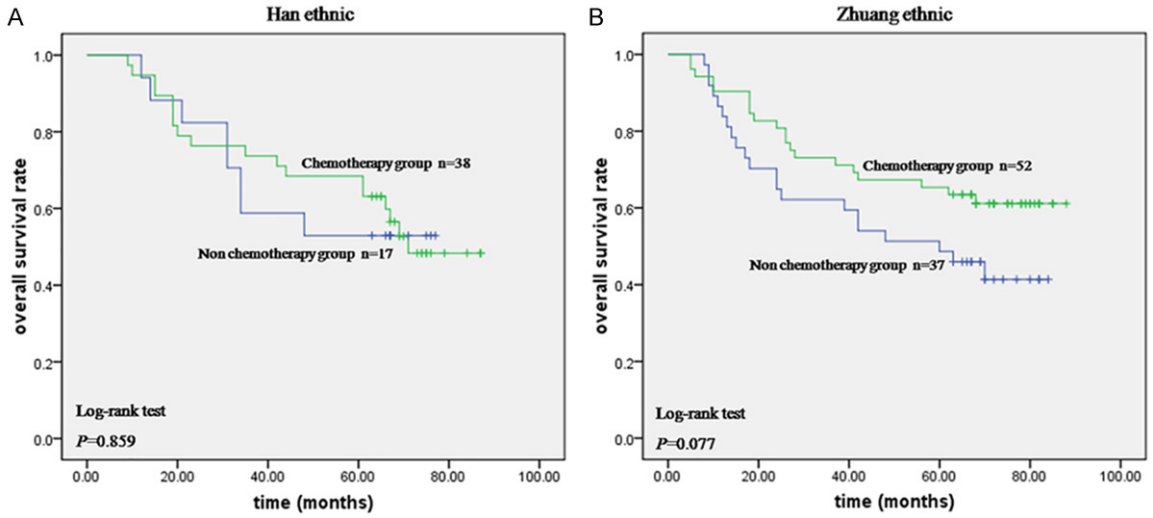

Figure 3. Effect of chemotherapy on the prognosis of NPC patients. A: The relationship between chemotherapy and OS time of Han NPC patients; B: The relationship between chemotherapy and OS time of Zhuang NPC patients.

Our results showed that the MTA proteins mainly expressed in the nuclei of NPC samples in both Han and Zhuang ethnicities. The correlation analysis of the MTA expression showed that the association among MTA1, MTA3 and expression in NPC tissues was different in Zhuang and Han ethnics. MTA1 expression was positively associated with MTA2 expression in both Han (r=0.320, P=0.034) and Zhuang (r=0.410, P=0.000) ethnicities. In Han ethnic group, MTA3 expression was positively correlated with MTA2 expression (r=0.344, P=0.018) but showed no significantly relationship with MTA1 expression (P=0.071). In contrast, MTA3 expression was not correlated with MTA2 expression (P=0.097) evidently but associated with MTA1 expression significantly (r=0.563, P=0.000) in Zhuang ethnic group. The correlation analysis between MTAs expression and clinical indexes showed that: in Han ethnic group, the expression of MTA1 was positively correlated with the N staging, and the expression of MTA2 had an inverse correlation with age, while the expression of MTA3 was positively correlated with gender. In Zhuang ethnic group, only the expression of MTA3 was positively correlated with age, recurrence and metastasis of NPC patients, while the expression of MTA1 and MTA2 had no significant correlation with the clinical indexes. The survival analysis showed that: in Han ethnic group, there was no clearly correlation between the MTA2 expression/MTA3 expression and the overall survival time. Only the patients with high MTA1 expression had worse prognosis (40.0% VS 88.9%, P=0.026), which was consistent with previous studies on the relationship of MTA1 expression and the prognosis of NPC. In Zhuang ethnic group, the expression of MTA1, MTA2 were not significantly correlated with the overall survival time. Only the patients with high expression of MTA3 had worse prognosis (38.9% VS 62.1%, P=0.031). Therefore, MTA1 (P=0.254) as well as MTA3 (P=0.294) could not be an independent predict factor.

Based on these results, we speculated that the biological functions of MTA protein family in NPC tissues were different from ethnics. The high expression of MTA1 might promote the metastasis and proliferation of NPC cells to regional lymph nodes and reduced the survival time of the Han NPC patients, but showed no significant association with the prognosis of Zhuang NPC patients. Conversely, MTA3 high expression might reduce the survival time of Zhuang NPC patients via promoting the metastasis, but not affecting the overall survival time of Han NPC patients significantly. In both Han and Zhuang ethnics, MTA2 did not perform significant influence on NPC patients' prognosis. Li and colleagues found that the expression of MTA1 was positively correlated with the N staging, M staging and clinical staging, and associated with poor prognosis of NPC patients as an independent predictive factor [15]. Yuan et al

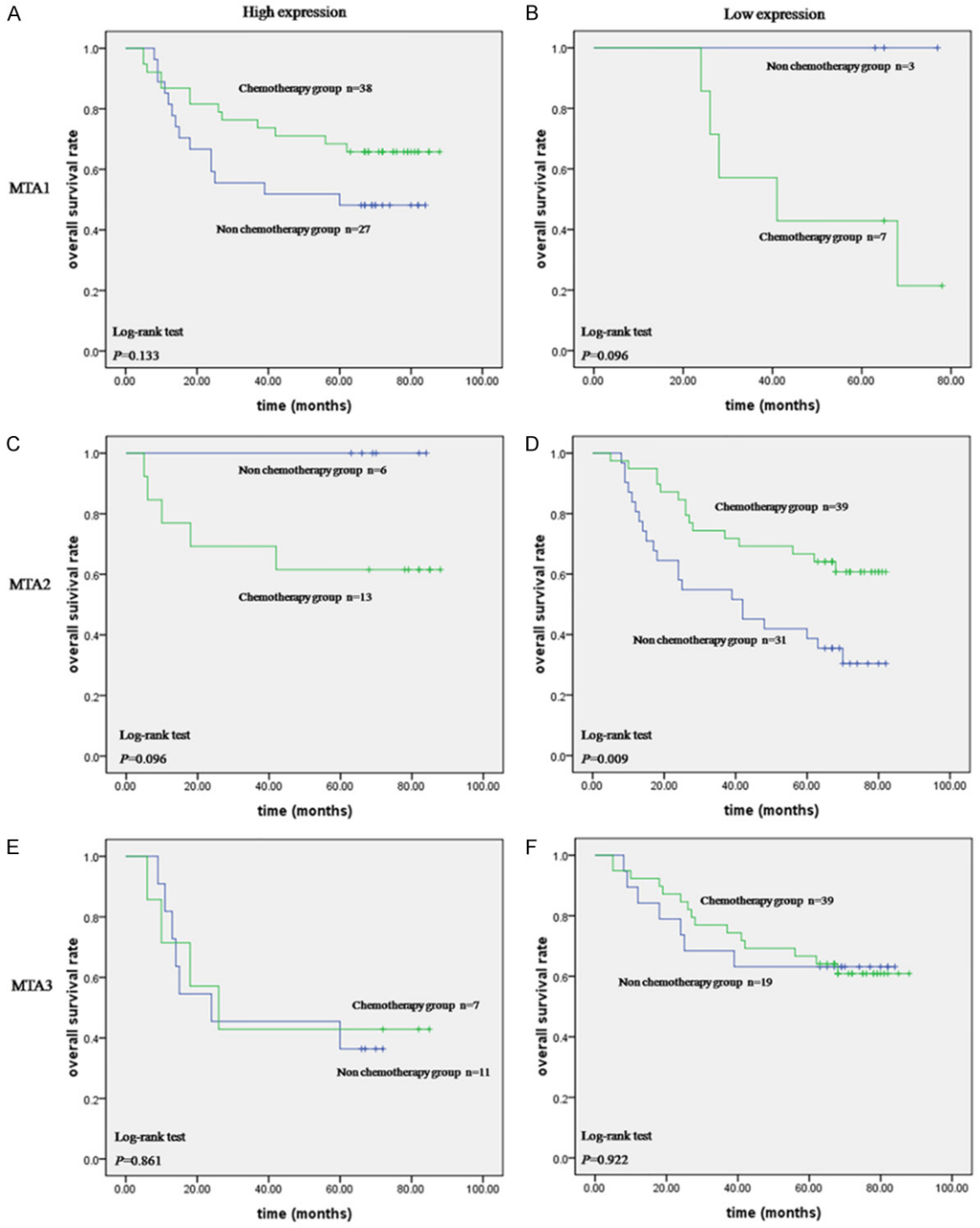

Figure 4. The correlation between chemotherapy and the prognosis of Zhuang NPC patients with different MTA expression. A: Chemotherapy could not significantly affect the OS of NPC patients with MTA1 high expression (65.8% vs 48.1%, P=0.133). B: Chemotherapy could not significantly affect the OS of NPC patients with MTA1 low expression (28.6% vs 100.0%, P=0.096). C: Chemotherapy could not significantly affect the OS of NPC patients with MTA2 high expression (61.5% vs 100.0%, P=0.096). D: Only in the MTA2 low expression subgroup, the prognosis of NPC patients with chemotherapy was significantly better (55.4% VS 35.4%, P=0.012). E: Chemotherapy could not completely affect the OS of NPC patients with MTA3 high expression (42.9% vs 36.4%, P=0.861). F: Chemotherapy could not completely affect the OS of NPC patients with MTA3 low expression (61.5% vs 63.2%, P=0.922).

found that the expression of MTA1 was positively correlated with the T staging, M staging and clinical staging, but had no correlation with the overall survival time and negatively correlated with the distant metastasis free survival time [7]. These articles had suggested the tumor promoting function of MTA1 in NPC, in consistent with our results of Han ethnic. Although the information of patients' ethnic was not given, the samples were collected from the hospitals of Guangzhou, which indicated that the study may be mainly in the Han population. Thus the conclusions of the two articles about the MTA1 function were similar with our results in the Han NPC patients, but differed from our study in Zhuang NPC patients [17]. This article found that the expression of MTA2 was positively correlated with the N staging and clinical staging, and up regulation of MTA2 could promote the proliferation, invasion ability of NPC cells. We hypothesized that the MTA2 gene expression in patients with NPC might be ethnic and regional specific. As a tumor metastasis-related protein, MTA2 is involved in metastasis and prognosis in various cancers. For example, the expression of MTA2 was closely related to the depth of tumor invasion, lymph nodes metastasis, and TNM staging in gastric cancer tissues. MTA2 knockdown impairs invasion and metastasis of gastric cancer cells, and attenuates xenografts growth in vivo [18]. Moreover, nuclear MTA2 was a risk factor of distant metastasis in patients with HCC andKi-67 showed a negative correlation with histological grade [19]. Liu et al found that MTA2 is usually overexpressed in ESCC cells and increased expression of MTA2 was associated with a poor prognosis, which suggest that MTA2 overexpression may play an important role in the progression of ESCC [10]. At last, our results showed that MTA3 was an oncogene in Zhuang ethnic. Previous studies on MTA3 had revealed its contradictory role in different tumors. For example, Li et al demonstrated that MTA3 was overexpressed in 57.4% non-small cell lung cancer, and positively correlated with T staging, local lymph node metastasis and clinical staging, and the patients with MTA3 high expression had worse prognosis [13]. However, the tumor suppress function of MTA3 had also been reported. For example, Dong et al found that the prognosis of patients with MTA3 high expression of gastroesophageal junction carcinoma was significantly better, and MTA3 was an independent predictive factor for this disease, as the expression of MTA3 was negatively associated with pathological grade, clinical stage, lymph node metastasis and distant metastasis [14]. All these results proved that MTA3 might be involved in different signaling pathways in different tumors, and acted different biological functions. To our knowledge, there was no relevant research report on MTA3 expression in NPC before. Based on our data, we hypothesized that MTA3 might promote the NPC recurrence or metastasis in Zhuang ethnic.

Taken together, MTA1, MTA2 and MTA3 might be involved in different gene regulation networks and had different biological functions of NPC patients in different ethnic groups and different regions. The MTA1 expression was associated with the prognosis of NPC patients in Han ethnic but not effected overall survival time of Zhuang NPC patients significantly. MTA2 showed no clearly influence on NPC patients' prognosis in Guangxi region. MTA3 was suggested to be an oncogene of NPC in Zhuang ethnic.

Our results also found that the chemotherapy regimen of "carboplatin + fluorouracil" did not affect the overall survival time in patients with NPC of Han ethnic obviously (52.6% VS 52.9%, P=0.859), but it could improve the overall survival time of Zhuang ethnic patients, although the correlation was not significantly (61.5% VS 43.2%, P=0.077). We then analyzed the effect of the chemotherapy regimen on the overall survival time of patients with NPC in the Zhuang ethnic with different MTA expression. The results showed that: only the patients with low expression of MTA2 (70 cases) had better prognosis (61.5% VS 32.3%, P=0.009). In other groups, there were no significant correlation between chemotherapy and prognosis (P> 0.05). It was worth noting that, due to the economic and other reasons, the proportion of NPC patients received chemotherapy in Zhuang ethnic was lower than that in Han ethnic, which was similar to the data of our experiment (58.24% VS 69.64%). Our results demonstrated that the Zhuang NPC patients especially with low MTA2 expression were more needed to receive chemotherapy than Han NPC patients. Further studywith larger number of NPC samples and other experimental methods were

indispensable for developing individualized chemotherapy of Zhuang NPC patients with low MTA2 expression, and establishing chemotherapy efficiency of Han NPC patients.

In conclusion, our experiments mainly studied the expression of MTA in NPC, and it was proved that this family had a certain tumor promoting function in patients with NPC, and biological functions might be ethnic differences. Most importantly, we found that the patients with MTA2 low expression in Zhuang ethnic can benefit from chemotherapy, which suggesting MTA2 to be an important marker for guiding clinical treatment of NPC. In the future, we plan to investigate more NPC samples and supplement cytology experiments to further study the gene regulatory network of MTA family and the molecular mechanism of MTAs expression on the efficacy of chemotherapy for guiding the clinical individualized treatment of NPC.

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Disclosure of conflict of interest

None.

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