Original Article
Research on correlation between MTA3 expression level and prognosis of pancreatic cancer

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Abstract: Objective: To studythe correlation between MTA3 protein and prognosis of pancreatic cancer. Method: The researcher made a tissue chip with 100 pancreatic cancer cases (interview information attached), then tested MTA3 expression with immunohistochemical method and analyzed correlation between experiment data and prognosis with SPSS software. Results: MTA3 expression in pancreatic cancer and adjacent tissues appears no obvious difference (P=0.134) yet apparent positive correlation, i.e. patients with high MTA3 protein expression in adjacent tissues also appear high MTA3 expression in cancer tissues (r=0.281, P=0.014). MTA3 expression in pancreatic cancer tissues appears negative correlation with patient's diabetes mellitus history (r=-0.264, P=0.036). The negative correlation between MTA3 expression in adjacent tissues and tumor size (r=-0.309, P=0.006) indicates mitigation effect of high MTA3 expression against tumor issues. Survival time analysis indicates that MTA3 expression in adjacent tissues has obvious positive correlation with prognosis (independent prognosis factor, P=0.002) of pancreatic cancer patients (35.5% to 6.3%, P=0.000) while MTA3 expression in tumor tissues appears no correlation with prognosis (26.7% to 25.0%, P=0.567). Conclusion: MTA3 expression in adjacent tissues has obvious correlation with prognosis of pancreatic cancer patients. Possibility exists in correlation between MTA3 expression in pancreas and diabetes mellitus development.

Keywords: MTA3, pancreatic cancer, diabetes mellitus, tissue chip, immunohistochemistry, prognosis

Introduction

Pancreatic cancer is a kind of tumor with severe malignance and bad prognosis. Most pancreatic cancer patients are died from tumor cell infiltration and metastasis [1]. Research about tumor cell infiltration and metastasis, as well as molecular mechanism of tumor cells is thus of particular importance to specific molecular target searching and pancreatic cancer treatment.

MTA (metastasis-associated gene) is a gene family with close relationship to tumor development, which includes products of MTA1, MTA2 and MTA3. Such family is mainly involved in composition of nucleosome remodeling histone deacetylase as inhibition against gene transcription. It also promotes tumor cell invasion and metastasis by regulating estrogen path, cytoskeleton and apoptosis. The high expression of MTA genes in several kinds of dermic tumor tissues has been proved in abundant of in vivo experiments, in vitro experiments and clinical researches [2]. Further researches indicate that MTA is of strong correlation with invasiveness, metastasis and prognosis of such tumors. For example, MTA1 may change intercellular junction and trigger nasopharyngeal cancer metastasis by influencing GTP enzyme through Hedgehog signal pathway [3]. As for prostate cancer cases, MTA1 may strengthen invasiveness of tumor cells by regulating E-cadherin expression through AKT signal pathway [4]. Nevertheless, researches on effect of MTA3 towards tumor generation and development are still short in amount. This research discusses about correlation between MTA3 and pancreatic cancer prognosis, which is useful for studying the molecular mechanism of MTA3 participating pancreatic cancer invasion and metastasis. It may further become a biological

sign for clinical seeking towards tumor prognosis and a new target for targeted therapy.

Materials and methods

Source of pancreatic cancer samples (tissue chip)

Pancreatic cancer tissue chip (HPan-Ade180-Sur-02) is made by Shanghai Outdo Biotech Co., Ltd which includes 100 samples of pancreatic cancer tissue and 80 samples of corresponding adjacent tissues (adjacent tissue indicates tissues within 1.5 cm from the tumor tissue).

Interview to pancreatic cancer patient: all interviewees accepted their surgery between September 2004 and December 2008. The last interviewee was interviewed in December 2011. All cases are pathologically diagnosed pancreatic cancer patients (mainly pancreatic ductal cancer) who have accepted no presurgical therapy.

Immunohistochemical experiment

Immunohistochemical experiment in two- step method: The tissues are treated with high- temperature high- pressure antigen retrieval, respectively added with first MTA3 antibody (1:500, 14682-1-AP, Proteintech Co., Ltd) after serum blocked, and placed overnight under 4°C. Then, the tissue will be applied to microscope observation and analysis after added with second antibody (rabbit anti-rabbit immunoglobulin (DAKO) labelled by HRP), PBS rinsing, DAB staining, dehydration, transparentized and mounted.

The observation takes 3 random high- power lens observations, and counts positive cells among no less than 3×100 cells. The calculation is about positive staining rate and staining intensity of positive cells among all tested cells. Scoring formula of positive staining rate: 0 (0%), 1 (1-25%), 2 (26%-50%), 3 (51-75%), 4 (76%-100%). Scoring formula of staining intensity: 0 (0), 0.5 (0-1+), 1 (1+), 1.5 (1+~2+), 2 (2+), 2.5 (2+~3+), 3 (3+). By adding "staining intensity score" to "positive staining score" of MTA3 nucleus, tissues score no larger than 4 are divided into low antibody expression group while those score larger than 4 are divided into high antibody expression group.

Statistical analysis

With failed experiment results excluded, all left 97 groups of pancreatic cancer immunohistochemical data and 78 groups of adjacent cancer tissue immunohistochemical data are involved in the statistical analysis.

The correlation research about MTA3 expression in pancreatic cancer tissues and adjacent tissues adopts paired Wilcoxon comparison experiment and Pearson correlation analysis. Research about correlation between MTA3 expression and clinical index of pancreatic cancer adopts Spearman's correlation analysis method. Single-factor correlation analysis between MTA4 and survival time of pancreatic cancer tissues adopts Kaplan-Meier survival analysis and log-rank statistical test while statistically meaningful variables in single-factor analysis will be involved with COX multi-factor regression survival analysis. P<0.05 indicates statistically meaningful.

Results

Correlation research about MTA3 expression in pancreatic cancer tissues and adjacent tissues

According to immunohistochemical data, MTA3 antibody expression mainly exists in nucleus of pancreatic cancer cells and adjacent tissues. It also appears small quantity of positive cytoplasm reaction which is consistent with expression positioning and antibody introduction of this protein.

After excluding failed experiment results, all left 97 groups of pancreatic cancer immunohistochemical data and 78 groups of adjacent cancer tissue immunohistochemical data are involved in paired Wilcoxon comparison analysis and Pearson correlation analysis. Analysis results indicate that: MTA3 expression appears no obvious difference in pancreatic cancer tissues and adjacent tissues (P=0.134) yet appears positive correlation, i.e. patients with higher MTA3 expression in cancer tissues are also found with high MTA3 expression in adjacent tissues (r=0.281, P=0.014). Analysis results are as shown in **Tables 1** and **2**. Pictures of typical immunohistochemical staining are as shown in **Figure 1**.

Table 1. Correlation of MTA3 expression in pancreatic cancer tissues and adjacent tissues

Category	Mean ± Std. Deviation	*P*-value
MTA3 expression in cancer tissues (76 groups)	1.880±0.329	0.134
MTA3 expression in adjacent tissues (76 groups)	1.790±0.406	

MTA3 expression in pancreatic cancer tissues and adjacent tissues appears no obvious difference (P=0.134).

Table 2. Correlation of MTA3 expression in pancreatic cancer tissues and adjacent tissues

	Parameter	MTA3 expression inadjacent tissues
MTA3 expression incancer tissues	Pearson Correlation	.281*
	Sig. (2-tailed)	.014
	N	76

Obvious positive correlation between MTA3 expression in pancreatic cancer tissues and adjacent tissues-patients with higher MTA3 expression in cancer tissues are also found with high MTA3 expression in adjacent tissues (R=0.281, P=0.014). *P<0.05, **P<0.01.

Correlation research about MTA3 expression and clinical index of pancreatic cancer

Spearman's correlation analysis indicates that: MTA3 expression in pancreatic cancer tissues and diabetes mellitus history of patients appear negative correlation (r=-0.264, P=0.036) while MTA3 expression in adjacent tissues and tumor size appears negative correlation (r=-0.309, P=0.006). Analysis results are as shown in **Table 3**.

Spearman's correlation analysis: P<0.05 indicates statistical difference, *P<0.05, **P<0.01; Correlation coefficient r: positive number indicates positive correlation, negative number indicates negative correlation. The closer absolute value is to 1, the stronger the correlation is.

Correlation between MTA3 expression and pancreatic cancer prognosis: single-factor survival time analysis

Interview to 100 pancreatic cancer patients: all interviewees accepted their surgery between September 2004 and December 2008; the last interviewee was interviewed in December 2011. During the interview cycle, 73 interviewees died from pancreatic cancer, with median survival time of 8 months (0 to 43 months); 27 interviewees are still alive, with median survival time of 47 months (36 to 87 months).

Single-factor survival time analysis is conducted with Kaplan-Meier survival analysis and log-rank statistical test. Analysis results indicate that: MTA3 expression in cancer tissues has no correlation with prognosis of pancreatic cancer patients (26.7% to 25.0%, P=0.567). MTA3 expression in adjacent tissues has obvious positive correlation with prognosis of pancreatic cancer patients (35.5% to 6.3%, P=0.000), i.e. pancreatic cancer patients with high MTA3 expression in adjacent tissues appear longer survival time and total survival rate obviously higher than patients with low MTA3 expression (50.0% to 16.7%). Detailed results are as shown in **Figure 2**.

COX multi-factor regression analysis

By involving statistically meaningful variables in single-factor analysis into COX multi-factor regression survival analysis, analysis results indicate that: MTA3 expression in adjacent tissues is an independent related factor to pancreatic cancer prognosis (P=0.002). Moreover, pathological grade is also an independent related factor to pancreatic cancer prognosis (P=0.001). Analysis results are as shown in **Table 4**.

Discussions

MTA3 is an important member of the MTA group. As a part of histone deacetylase, it is an important regulating factor in epithelial-mesenchymal transition (EMT) which has critical effect in development and progress of gastro-esophageal junction adenocarcinoma [5]. Researches about MTA3 and tumors indicate that MTA3 has different, even contrary effect towards different tumors. For example, some researches have reported that MTA3 expression is down-regulated in senior endometrioid adenocarcinoma, which indicates MTA3 may become an inhibition factor in such cancer tissues. In breast cancer tissues, MTA3 has negative correlation with invasiveness and metastasis of tumors, which indicates that MTA3 also works as inhibition factor in breast cancer. However, MTA3 appears high expression in
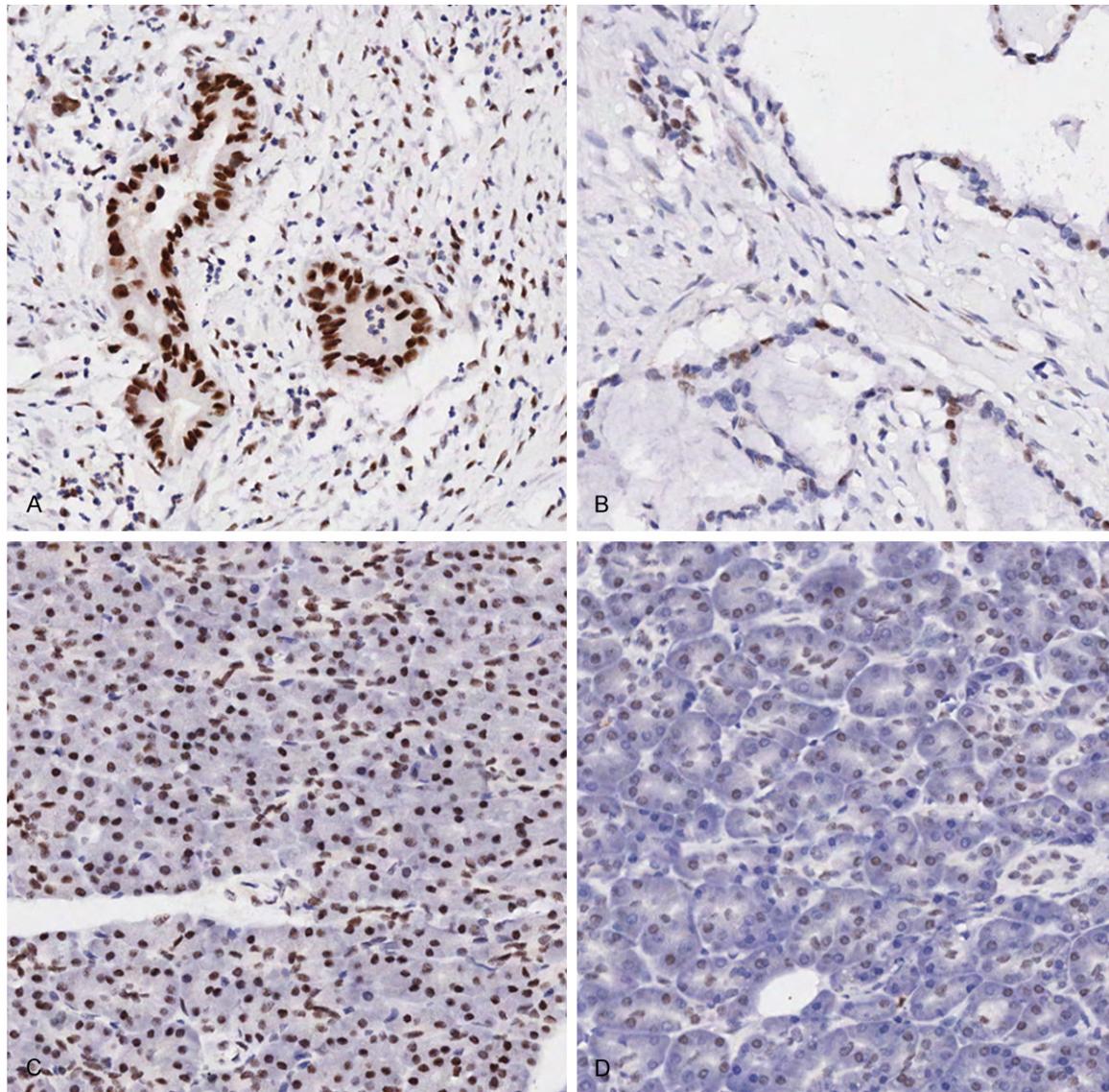
Figure 1. Immunohistochemical experiment results of MTA3 protein: high expression in Cancer Tissue A, low expression in Cancer Tissue B, high expression in Adjacent Tissue C, low expression in Adjacent Tissue D (enlargement multiple: ×200).

Table 3. Correlation research about MTA3 expression and clinical pancreatic cancer indexes

Category	Parameter	Gender	Age	Tumor size	Grade	T	N	M	Clinical stage	History of diabetes
MTA3 expression (cancer tissue)	Correlation Coefficient	-.093	-.085	-.046	-.011	-.040	-.103	.110	-.116	-.264*
	Sig. (2-tailed)	.364	.410	.660	.914	.701	.332	.284	.270	.036
	N	97	97	95	97	95	91	97	93	63
MTA3 expression (adjacent tissue)	Correlation Coefficient	.013	-.124	-.309**	-.044	-.118	-.083	-.045	-.166	-.084
	Sig. (2-tailed)	.909	.279	.006	.700	.307	.485	.699	.158	.534
	N	78	78	77	78	77	73	78	74	57

non-small cell lung cancer. Such overexpression is highly positively related to clinical stages and lymph node metastasis, but also worse prognosis of patients with high MTA3 expression [7]. Biological function of MTA3 in pancreatic cancer cases and correlation between
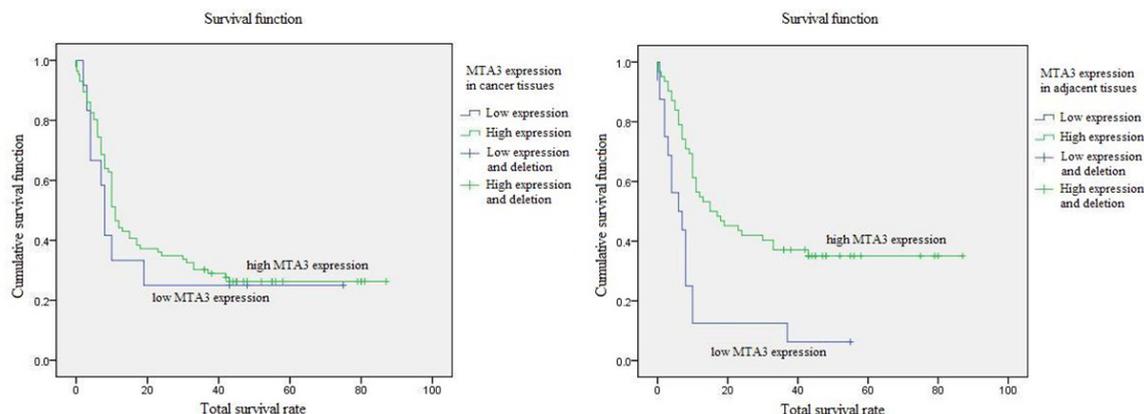
Figure 2. MTA3 expression in cancer tissues has no correlation with prognosis of pancreatic cancer patients (26.7% to 25.0%, P=0.567). MTA3 expression in adjacent tissues has obvious positive correlation with prognosis of pancreatic cancer patients (35.5% to 6.3%, P=0.000), i.e. pancreatic cancer patients with high MTA3 expression in adjacent tissues appear longer survival time and total survival rate obviously higher than patients with low MTA3 expression (50.0% to 16.7%).

Table 4. Independent pancreatic cancer prognosis-related factor analysis with COX multi-factor regression method

Factor	B	SE	Wald	df	P-value	Exp (B)	95.0% CI for Exp (B)	
							Lower	Upper
Pathological grades	.800	.248	10.429	1	.001	2.225	1.369	3.615
N	.877	.502	3.050	1	.081	2.404	.898	6.433
Clinical stages	.335	.512	.427	1	.514	1.397	.512	3.814
MTA3 expression in adjacent tissue	-1.112	.355	9.804	1	.002	.329	.164	.660

SE: standard error; DF: degree of freedom; CI: confidence interval; Lower: lower limit; Upper: upper limit.

MTA3 and pancreatic cancer prognosis are not reported.

In order to study correlation between MTA3 and generation, development, metastasis and prognosis of pancreatic cancer, this research has conducted immunohistochemical and statistical analysis on 100 samples of pancreatic cancer tissues with consummated clinical information and attached interview record so as to learn about expression and clinical significance of such protein in pancreatic cancer. Analysis results indicate that: MTA3 expression appears no obvious difference in pancreatic cancer tissues and adjacent tissues (P=0.134), yet MTA3 expression in pancreatic cancer tissues is positively correlated to MTA3 expression in adjacent tissues, i.e. patients with high MTA3 expression in cancer tissues also appear high MTA3 expression in adjacent tissues (r=0.281, P=0.014). Such positive correlation implies that MTA3 may be involved in similar signal

pathway and has similar biological function in both cancer tissues and adjacent tissues. Correlation analysis proves that: MTA3 expression in pancreatic cancer tissues is obviously negatively correlated to diabetes mellitus history (r=0.264, P=0.036); pancreatic cancer patients with diabetes mellitus history appear lower MTA3 expression in their cancer tissues. Patients who appear higher MTA3 expression are with smaller tumors, which indicate that high expression of MTA3 in adjacent tissues may inhibit the tumor. Following survival time analysis further proves such conclusion: MTA3 expression in adjacent tissues is both positively correlated to prognosis of pancreatic cancer patients (35.5% to 6.3%, P=0.000) and an independent prognosis factor (P=0.002), while MTA3 expression in cancer tissues has no correlation with prognosis of pancreatic cancer patients (26.7% to 25.0%, P=0.567). Moreover, pathological grade of tumors is also an independent prognosis factor to pancreatic cancer

patients (P=0.001). In conclusion, our researches indicate that: MTA3 possibly takes similar signal pathway, and has similar inhibition effect against cancer in both cancer tissues and adjacent tissues. Patients with high MTA3 expression in adjacent tissues appear not only obviously smaller tumor size, but also apparently better prognosis.

Abundant researches prove that "tumor microenvironment" composited of tumor cells, adjacent epithelial cells (adjacent tissue), fibroblast, innate immune cells, peculiar immune cells, tumor vessels, constitutive cell of lymphatic tissues, mesenchyma with tissue specificity, expression product and metabolic substance [8] is of significant effect to generation and development of tumors. Some researches have proven that immunological factors within the tumor microenvironment are the most accurate independent prognosis factor which is even more precise than TNM stages [9]. For example, liver cancer research conducted by WANG Guangzhen and her colleagues [10] indicates that: liver cancer patients with larger tumor size have more mast cells in adjacent tissues (P=0.04); amount of macrophage within adjacent tissues is of negative correlation with total survival rate (P=0.003) while amount of mast cell in adjacent tissues is of negative correlation with disease-free survival rate (P=0.002). Both two amounts are independent predictor. In combination of our test results, it is reasonable to speculate that MTA3 is involved in immunological signal network of pancreatic cancer tissue's tumor environment, while high MTA3 expression within cancer tissues, especially adjacent tissues inhibits amount of macrophage, mass cell and other inflammatory factors which further mitigates inflammatory damages and promotes prognosis. Our test has also proven that MTA3 expression in pancreatic cancer tissues is of obvious correlation with diabetes mellitus history. The fact that pancreatic cancer patients with diabetes mellitus history appear lower MTA3 expression implies that low MTA3 expression is of certain correlation with diabetes mellitus. Pathogenic molecular mechanism of diabetes mellitus is complicated, but β islet cells damaged by macrophage and other inflammatory factors generated by immune disorder is the most important pathogenic mechanism [11]. Considering correlation between diabetes mel-

litus and pancreatic cancer [12], it is reasonable to speculate that MTA3 has already become protection when patients appear pancreatic immunity damage instead of early pancreatic cancer stage. High MTA3 expression inhibits inflammatory factors and further prevents diabetes mellitus by avoiding inflammatory damage to pancreas, while patients with low MTA3 expression are more possible to be sickened by diabetes mellitus for lacking such protection. Meanwhile, such protection mechanism of MTA3 towards normal pancreatic cells also has limited inhibition effect in development process of pancreatic cancer.

Researches toward immune regulation mechanism of pancreatic cancer have proven that the NF-κB activated in human pancreatic cancer cells is of critical influence in generation, development, anti- apoptosis, infiltration, metastasis and vessel generation of the tumor [13]. Researches about correlation between macrophage, mass cells and pancreatic cancer indicates that macrophage and mass cells within interstitial substances of pancreatic cancer tissues are involved in tumor vessel development, tumor microenvironment establishment, and promotion toward infiltration and metastasis of pancreatic cancer cells [14]. Researches about correlation between the MTA group and immunological signal network are short in amount, and mainly focus on MTA1. Ghanta and his colleagues found in their research that MTA1 may trigger expression of macrophage, mass cells and other inflammatory factors, inhibit regeneration capacity of normal epithelial cells, promote tumor cell development and metastasis, and degrade prognosis of patients by regulating TG2 expression (transglutaminase 2) and starting the NF-κB signal pathway. Report about MTA3 and immunological signal pathway is rarely found. We speculate that MTA3 may have contrary immunological regulation mechanism contrary through similar methods with MTA1, which mitigates inflammatory damages caused by macrophage and mass cells and obviously promotes prognosis of patients. Besides, absence of MTA3 expression in pancreas may be correlated to generation and development of diabetes mellitus and further related to pancreatic cancer.

So far, our research, to a certain extent, has proven correlation between MTA3 and pancre-

atic cancer prognosis, but still lacks knowledge about related molecular mechanisms. Our next step is to conduct allogenetic pancreatic cancer cell seeding of MTA3+ and MTA3- on nude mice to acquire further understanding about molecular mechanism of MTA3 in generation and development of pancreatic cancer.

Disclosure of conflict of interest

None.

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