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Original Article

Expression of HDGF, ADAM9 and P53 is correlated with clinical and pathological features and prognosis of esophageal cancer

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Abstract: Objective: To explore the relationship between the clinical features of esophageal cancer and the expression level of HDGF, ADAM9 and P53, as well as the interrelation among expression levels of HDGF, ADAM9 and P53. Methods: HDGF, ADAM9 and P53 expression in 118 patients with esophageal cancer (81 males and 37 females) with ages ranging from 46 to 84 years old (with median age of 60 years old) were analyzed by immunohistochemistry. The expression levels of ADAM9, HDGF and P53 in cancer and normal specimens were stratified into two levels. Level 1 represents absent expression and level 2 represents presence of protein. The experimental group of cancer samples was then compared to the control group of normal specimens. Results: There was significant difference in HDGF expression between the esophageal cancer group and the normal control ($P<0.05$). In patients with esophageal cancer, level 2 expression of HDGF, ADAM9 and P53 is found more frequently in in large tumors, cases with lymph node metastasis, cancer of advanced stages and Five-year mortality rate in comparison to cases with level 1 expression. In addition, ADAM9 expression is associated with that of HDGF, and was showing a positive correlation according to spearman correlation coefficient. on the other side, expression of P53 had a negative correlation with that of ADAM9 and HDGF. Conclusions: HDGF, ADAM9 and P53 expression levels were shown to be prognostic factors for esophageal cancer. Expression of P53 has a negatively correlation with expression of HDGF and ADAM9, and there was a positive correlation between expression of HDGF and ADAM9.

Keywords: HDGF, ADAM9, negative correlation, positive correlation, immunohistochemistry, P53, esophageal cancer

Introduction

The family of A disintegrin and metalloprotease (ADAM) is involved in different pathogenic and physiological functions including cell adhesion, cell migration, and tissue remodeling [1-3]. One of the ADAM proteins, ADAM9, is found to be a critical regulator of cell-cell signaling. It is also known to play an important role in pathogenic processes. Various ADAM family members have been shown to be potentially important diagnostic and prognostic markers in human malignancy [4]. ADAM9 As a potential target molecule in cancer, ADAM9 is consistently over-expressed in various human cancers, such as pancreatic ductal adenocarcinoma (PDAC), non-small cell lung cancer, gastric cancer, and plays an important role in tumorigenesis in mouse models [5].

Hepatoma derived growth factor (HDGF), a secreted heparin-binding growth factor, was originally isolated from the human hepatoma cell line Huh-7 [6]. It has been implicated in cancer initiation and progression. It has been identified that HDGF stimulates cell growth after translocation to the nucleus [7]. Like ADAM9, recent research confirmed that the expression level of HDGF was elevated in many human cancers [8].

Matsuyama A. et al. studied esophageal cancer and they demonstrated that HDGF played an important role in radiosensitivity. They reported that radiotherapy was more effective in clinical cases with high HDGF mRNA expression in comparison to cases with low expression. Therefore, it was proposed HDGF could be a novel marker predicting effectiveness of radiotherapy in clinical cases [9].

As a well-established tumor suppressor since first discovered in 1979, p53 plays a pivotal role in suppressing tumorigenesis through inducing cell cycle arrest and/or apoptosis [10-

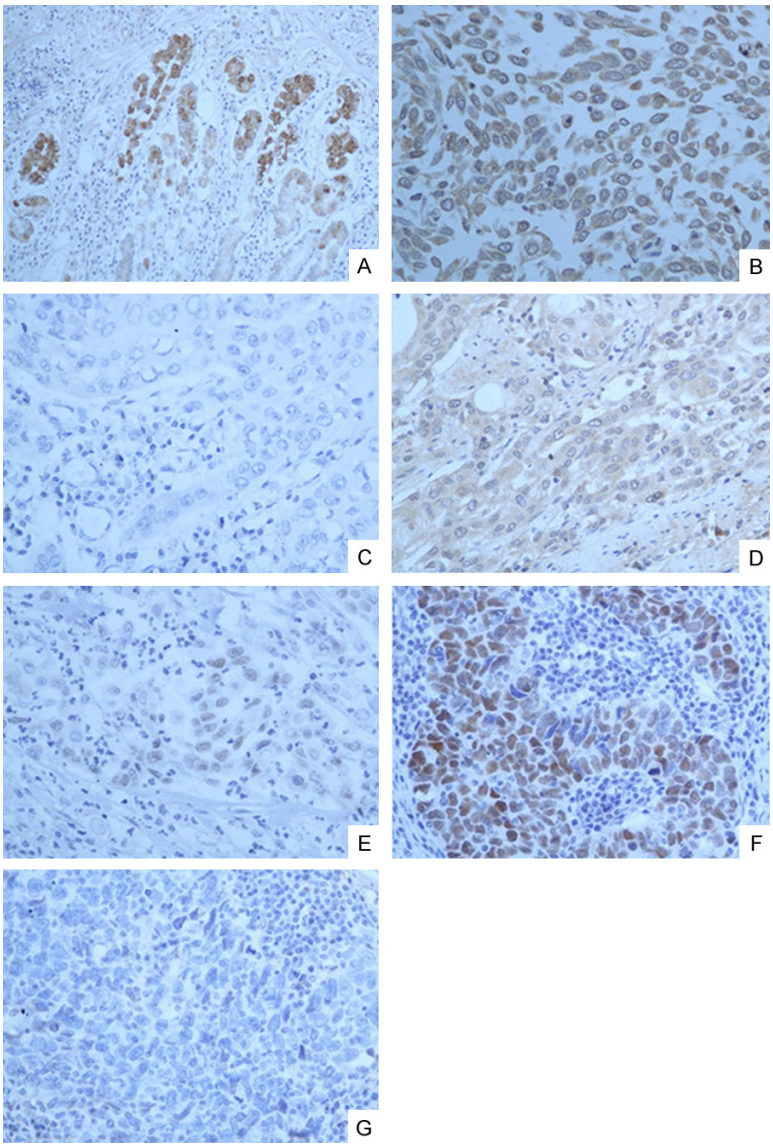

Figure 1. A and B. Level 2 of ADAM9 expression in EC (A: + B: ++, magnification, ×400), the positive expression was located in the cytoplasm or cell membrane; C and D. Level 2 of HDGF expression in EC(C : + D :++, magnification, ×400), the positive expression was located in nucleus; E and F; Level 2 of P53 expression in EC (E: + F : ++, magnification, ×400), the positive expression was located in nucleus; G. Level 1 expression of what protein (need to clarify) (magnification, ×400).

Table 1. Comparison of ADAM9, HDGF and P53 expression in EC tissue and normal tissue

Groups	Cases No.	ADAM9			HDGF			P53		
		(-)	(+)	(++)	(-)	(+)	(++)	(-)	(+)	(++)
EC	118	25	24	69	27	26	55	43	45	55
control	10	7	2	1	6	2	2	0	1	9
χ^2 Value		24.000			16.000			24.000		
P Value		0.004			0.014			0.004		

12]. P53 was found to be mutated in approximately half of all human cancers [13], leading to aberrant cell growth. As a tumor suppresor, p53 is activated following DNA damage [14], hypoxia and other harmful stimuli to cells [15].

Sasaki Y et al. reported that HDGF was a critical target regulated by the tumor suppressor p53 [16, 17]. Endogenous HDGF expresion was decreased in cancer cells with introduction of wild-type p53; in addition, activated p53 downregulated HDGF expression after DNA damage. altogether, it w as shown that p53 negatively regulated the expression of HDGF [16]. Furthermore, it has been cofirmed that HDGF and ADAM9 are both over-expressed in various human cancers andtheir expression is closely corrlaed with with each other. HDGF and ADAM9 could bothbe considered as prognostic markers in lung cancer. However, the expression and interaction among HDGF, ADAM9 and p53 in esophgeal cancer, is still poorly defined. Here, we investigated the role of HDGF, ADAM9 and p53 in esophagealcancertogaina beter understanding of their relationship and more insight into their prognostic and therapeutic value in esophageal cancer.

Materials and methods

Patients and tumor specimens

Esophageal cancer (EC) speimens were randomly obtained from 118 patients who were diagnosed at Qilu hospital (Jinan, China) with esophageal cancer based on preoperative diagnostic exami-

Table 2. Correlation of expression of HDGF, ADAM9 and P53 with clinical and pathological features in esophageal cancer

Clinicopathological factors		Cases No.	ADAM9				HDGF				P53			
			(-)	(+)	(++)	*P* Value	(-)	(+)	(++)	*P* Value	(-)	(+)	(++)	*P* Value
Gender	Male	81	18	15	48	0.749	24	15	42	0.200	28	35	18	0.219
	Female	37	7	9	21		13	11	13		15	10	12	
Age(year)	<60	40	12	9	19	0.165	12	10	18	0.865	11	19	10	0.256
	>=60	78	13	15	50		25	16	37		32	26	20	
Tissue type	Squamous	105	22	23	60	0.481	33	21	51	0.276	39	41	25	0.518
	Adenocarcinoma	13	3	1	9		4	5	4		4	4	5	
Location	Upper	22	6	6	10	0.534	4	7	11	0.586	6	8	8	0.216
	Middle	53	10	8	35		18	10	25		22	23	8	
	Lower	43	9	10	24		15	9	19		15	14	14	
Tumor size(mm)	<40	63	17	16	30	0.037	24	18	21	0.008	21	22	20	0.241
	≥40	55	8	8	39		13	8	34		22	23	10	
Differentiation	High	36	12	7	17	0.055	11	10	15	0.126	10	13	13	0.234
	Moderate	40	10	6	24		17	4	19		13	18	9	
	Low	42	3	11	28		9	12	21		20	14	8	
Clinical stage	pT 1, pT 2	75	20	18	37	0.027	30	19	26	0.002	21	28	26	0.004
	pT 3, pT 4	43	5	6	32		7	7	29		22	17	4	
Lymph node metastasis	Negative	59	18	11	30	0.045	21	17	21	0.045	15	22	22	0.005
	Definite	59	7	13	39		16	9	34		28	23	8	
5-year survival or not	Yes	33	12	10	11	0.002	13	11	9	0.026	7	10	16	0.001
	No	85	13	14	58		24	15	46		36	35	14	

tions, incluing endoscopy, esophagography, computed tomography, and endoscopic ultrasonography in 2006. Histological diagnosis of EC was confirmed with biopsy specimens obtained before surgery and the pathological diagnosis were all squamous cell carcinoma or adenocarcinoma without distant metastasis. Besides, we took 10 normal esophageal specimens (the normal tissue next to cancer from surgical resection) as control.Tumor stage evaluations were based on the International Union Against Cancer pTNM classification.

Immunohistochemistry

Specimens of esophageal cancer were fixed in 10% formalin and processed for paraffin embedding. Histological sections were cut at 4 um. Rabbit-anti-human ADAM9, HDGF and P53 monoclonal antibody were obtained from Beijing Biosynthesis Biotechnology Co., LTD. All experimental procedures were followed based on the product manual strictly.

To investigate the expression levels of ADAM9, HDGF and P53 protein, we conducted the polyclonal antibodies (anti- ADAM9 antibody 1:400, anti- HDGF antibody 1:400, anti- P53 antibody 1:400) by immunohistochemistry SP method under the manufacturer's instruction.

To analyse immunohistochemical findings, five random fields were selected at 400X magnification under light microscope, the staining intensity was assessed and measured with Biosens Digital Imaging System. The positive expression of ADAM9 was located in the cytoplasm or cell membrane, and expression of HDGF and P53 were mainly observed in the nucleus. Tumor cells showing nuclear staining that was equal to or stronger than the staining intensity in vascular endothelial cells were considered nuclear positive. The same process was followed for cytoplasmic staining. Staining intensity of specimens was graded as - (absent, <10%), + (weak, 10%~50%), or ++ (strong, ≥50%) according to the proportion of positive cells in a random field as described in **Figure 1A**-**G**.

The expression level of ADAM9, HDGF and P53 was divided into two levels. Level 1 represents absent expression and level 2 represents both weak and strong expression.

Statistical analysis

SPSS software (Version 18.0) was used for all statistical analyses. The χ^2 test and Fisher's exact probability test were used to analyze the

Table 3. Relationship between the Expression of ADAM9 and HDGF in EC

ADAM9	HDGF			Total
	(-)	(+)	(++)	
(-)	13	2	10	25
(+)	11	7	6	24
(++)	13	17	39	69
Total	37	26	55	118
P Value			0.004	
Spearman correlation coefficient			0.278	

Table 4. Relationship between the Expression of ADAM9 and P53 in EC

ADAM9	P53			Total
	(-)	(+)	(++)	
(-)	4	9	12	25
(+)	6	8	10	24
(++)	33	28	8	69
Total	43	45	30	118
P Value			0.001	
Spearman correlation coefficient			-0.382	

Table 5. Relationship between the Expression of HDGF and P53 in EC

HDGF	P53			Total
	(-)	(+)	(++)	
(-)	10	13	14	37
(+)	6	11	9	26
(++)	27	21	7	55
Total	43	45	30	118
P Value			0.023	
Spearman correlation coefficient	-0.280			

relationship among ADAM9, HDGF and P53 expression and clinicopathologic variables of prognosis. Statistical significance was identified as $P < 0.05$.

Results

Patient outcome

After 5 years follow-up, 33 patients were still alive after surgery. The five-year overall survival rate was 27.97%.

Expression of HDGF, ADAM9 and P53 in esophageal cancer

HDGF expression was observed mainly in the nucleus in esophageal cancer. In contrast, the staining pattern in the normal esophageal specimens wasmore variable. Eighty-one cases (68.64%) showed staining in the nucleus of tumor cells, and were thus determined as nucleus-positive. Among them, twenty-six showed weak expression (32.10%), Fifty-five showed strong expression (67.90%). The remaining 37 cases (31.36%) was graded as absent expression, in which the proportion of positively stained cells was <10%.

However, ADAM9 and P53 expression wereobserved mainly in the cytoplasm and/or cell membrane, instead of nuclei, in esophageal cancer, as shownin **Table 1**.

Association between clinicopathologic features and ADAM9, HDGF and P53 expression in esophageal cancer

Association between HDGF expression and clinical factors is listed in **Table 2**. In comparison to esophageal cancer with HDGF level 1 expression,esophageal cancer with level 2 expression of HDGF exhibit more adverse clinical features in the following categories: presence of lymph node metastasis, tumor stage, and five-year overall mortality rate.

Consistently, ADAM9 expression is also correlated with adverse features of esophageal cancer. However, P53 did not show such degree of correlation. as presented in **Table 2**.

Relationship among ADAM9, HDGF and P53 expression in esophageal cancer

We also studied the relationship among ADAM9, HDGF and P53 expression in esophageal cancer (**Tables 3**-**5**). As shown in **Table 1**, ADAM9 had a close relationship with HDGFand was showing a positive correlation according to spearman correlation coefficient. In contrast, P53 showed a negative correlation with ADAM9 and HDGF, and the spearman correlation coefficients were -0.382 and -0.280, which indicated that P53 may negatively regulate the expression of ADAM9 and HDGF in EC, consistent with the findings from Sasaki Y. et al [16].

Discussion

HDGF is a unique growth factor and plays a critical role in the development and progression of carcinomas [18]. According to Yamamoto, the expression level of HDGF was highly

related to recurrence and prognosis of gastric carcinoma. They concluded that HDGF expression level was shown to be a prognostic factor in gastric carcinoma [6].

In our study, there were significant differences between the EC group and the normal control in HDGF expression ($P<0.05$). Cases with HDGF level 2 expression exhibited adverse clinical features including large tumor size, lymph node metastasis, tumor stage and five-year overall mortality rate in comparison to cases with level 1 expression. Our study suggested that HDGF expression isinvolved in EC progression. there was no significant correlation of HDGF level 2 expressions with age, gender, tumor location, histological type.

Consistent with what is observed from HDGF, ADAM9 expression is also correlated with adverse features of esophageal cancer. However, P53 did not show similar correlation.

Our research demonstrasted that a negative correlation existed between the expression level of P53 and the other two molecules, HDGF and ADAM9, which were closely correlated with the pathologic factors including tumor size, lymph node metastasis, tumor stage and five-year overall mortality rate. No significant difference was found between the P53 expression level and features including age, gender, tumor location, histological differentiation and type of tissue.

We also further explored the relationship between each pair of proteins: ADAM9 and HDGF, ADAM9 and P53, HDGF and P53, which were listed in **Tables 3**-**5**. We found that in cases where P53 expression level was strong, the ADAM9 and HDGF expression levels were low. This was similar to the result Sasaki Y. et al. reported in their study [16]. They concluded that p53 negatively regulated the HDGF expression [9, 16]. Here, our study showed that P53 might negatively regulate the HDGF and ADAM9 in EC. However, the expression level between HDGF and ADAM9 showed close correlation. This interesting correlation in expression levels of p53, HDGF and ADAM9 suggested that p53 negatively regulated expression of HDGF and ADAM9; further study is underway to clarify the prognostic and therapeutic role of the expression of these important proteins.

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Disclosure of conflicts of interest

None.

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