Int J Clin Exp Med 2013;6(8):700-705
www.ijcem.com /ISSN:1940-5901/IJCEM1307011

Original Article

MMP-2 and DcR3 expression in esophageal cancer tissue and correlation with patient survival

Ran Ao, Ya-Qi Du, Ying Wang, Yu-Shuai Chen, Bing-Yuan Wang

Department of Gastroenterology, The First Affiliated Hospital of China Medical University, Shenyang, China

Received July 18, 2013; Accepted August 11, 2013; Epub September 1, 2013; Published September 15, 2013

Abstract: Objective: This study aims to explore the expression of decoy receptor 3 (DcR3) and the matrix metalloproteinase-2 (MMP-2) in esophageal carcinoma and their relationship with patient survival. Methods: The EnVision™ immunohistochemistry method was used to examine DcR3 and MMP-2 expression in 150 surgical biopsies of esophageal carcinoma. Expression level was compared with clinical indices and with patient survival. Results: In cancer tissues, the positive expression rate of DcR3 and MMP-2 was 54.00% and 54.67% respectively; this was higher than levels in adjacent normal tissue. DcR3 and MMP-2 were positively correlated with carcinoma size, lymphatic metastasis, invasion degree, clinical stage and 3-year survival. DcR3 and MMP-2 were not correlated with gender, age and tumor degree of differentiation. DcR3 and MMP-2 were positively correlated between in the two groups ($r = 0.37$, $P < 0.01$). Kaplan-Meier survival curve showed that higher rates of DcR3 and MMP-2 expression correlated with lower survival. Conclusions: Determining DcR3 and MMP-2 expression may be useful for the diagnosis, treatment and prognosis of patients with esophageal carcinoma.

Keywords: Esophageal cancer, matrix metalloproteinase-2, decoy receptor

Introduction

Esophageal cancer (EC) is a common malignant tumor in the human digestive tract; its global incidence ranks 8th and its mortality ranks 6th among tumors. Advances in molecular biology reveal tissue biological markers that can affect tumor diagnosis, treatment, and prognosis [1]. Matrix metalloproteinase-2 (MMP-2) is a proteolytic enzyme that can degrade extracellular matrix components. MMP-2 plays an important role in invasion and metastasis of breast cancer, liver cancer and other malignant tumors, but its function in the occurrence of esophageal cancer is not clear. A decoy receptor (DcR) is a receptor that binds a ligand, inhibiting it from binding to its normal receptor and its related, downstream signal transduction. As such, decoy receptor regulates cell activity, differentiation and immunity by competing with functional receptors for their typical ligands [2-4]. Decoy receptor 3 (DcR3) is a newly discovered member of tumor necrosis factor receptor (TNFR) family; it is a soluble secretory protein lacking a transmembrane sequence [5]. In this study, immunohistochemistry was used to compare MMP-2 and DcR3 expression in esophageal cancer tissues and normal adjacent tissue, and their relevance to patient survival.

Materials and methods

Study subjects

Subjects ($N = 150$) were patients who received surgery in our hospital from January 2010 to December 2011. All study subjects had not received any preoperative chemotherapy or radiotherapy. All surgical specimens were fixed with 4% formaldehyde, paraffin-embedded and cut into 4 um sections. Cases of normal esophageal tissue specimens ($N = 30$) were taken > 5 cm away from tumors and were used as healthy controls. These 150 patients included 96 males and 54 females and the ratio of male and female was 1.78:1. The age range was 33-81 years with a mean age of 58.1 years. The patients were staged according to International Esophagus Cancer Staging Criteria by UICC,

with 77 cases of stage I/II and 73 cases of stage III/IV.

Detection methods

DcR3 and MMP-2 staining was completed with EnVision™ immunohistochemistry methods. MMP-2 antigen retrieval with 1 mmol/L EDTA buffer solution (pH = 9.0) water bath heat retrieval, and DcR3 antigen retrieval with 0.01 mmol/L citric acid buffer solution (pH = 6.0) microwave retrieval. The working concentrations of DcR3 and MMP-2 primary antibody were 1:150 and 1:40, respectively, and slides were placed in a wet box at 4°C overnight. Esophageal cancer positive sections were used as positive control, and PBS was used as negative control instead of the primary antibody. Mouse anti-human MMP-2 monoclonal antibody was purchased from Leica (Germany), mouse anti-human DcR3 monoclonal antibody was purchased from Abcam (Cambridge, MA USA), and the second antibody and color reagent DAB were from DAKO (Denmark). Assays were conducted according to manufacturers' instructions.

Result determination

The histology section results were read by two persons blinded to subject clinical status. Five fields on each sample were randomly selected and examined each section under high-power light microscope. Recorded data included total number of tumor cells and positive cells and the intensity of color development. The staining intensities ("I") were divided into 4 grades as defined by Gatalica: 0 for no staining, 1 for light staining, 2 for moderate staining, and 3 for strong staining. Percentage of positive cells ("P") was defined as: 0 for < 5%, 1 for 5% ~ < 25%, 2 for 25% ~ < 75%, 3 for ≥ 75%. The typological scores (H = I × P) was defined as: 0-1 as negative (-), 2-3 as weakly positive (+), 4-6 as moderately positive (++), and > 6 as strongly positive (+++). DcR3 and MMP-2 expression (-) was grouped to negative expression group, and DcR3 and MMP-2 expression (+), (++), and (+++) were grouped to positive expression group.

Follow-up

The follow-up information was obtained from telephone visit and clinic examination. All cases had complete follow-up data. The follow-up period was started from the diagnosis confirmation date. The survival rate was calculated from surgery date to the follow-up cutoff date (December 2011), or the date of death.

Statistical analysis

Statistical analysis was performed with SAS 9.1 statistical software. The enumeration data was expressed in rate, and the measurement data was expressed in mean values. χ^2 test or Fisher's exact test was used to compare between different groups, and Kendall correlation analysis method was used to performed correlation analysis. Inter-group survival rates were compared using Kaplan-Meier survival curves or Log-rank test; $P < 0.05$ was considered as statistically significant.

Results

MMP-2 and DcR3 expression in esophageal cancer tissues

MMP-2 was expressed in the intercellular space and cytoplasm of tumors, usually manifested as yellow, claybank particles. Among the 150 cases of esophagus cancer tissues, 81 cases showed positive MMP-2 expression with a positive expression rate of 54.00%, and 5 cases of normal adjacent tissues showed positive expression with a positive expression rate of 16.67%. The chi-square test showed a statistically significant difference in MMP-2 protein expression between esophageal cancer tissues and normal adjacent tissue ($\chi^2 = 13.97$, $P < 0.01$).

DcR3 was expressed in the cytoplasm and the cytomembrane of esophageal cancer cells, cancer cells at tumor margins were stained more heavily, and no expression was seen in interstitial cells. Among the 150 cases of esophageal cancer, 82 cases showed positive DcR3 expression with a positive expression rate of 54.67%; 8 cases of normal adjacent tissues showed positive DcR3 expression with a positive expression rate of 16.67%. The chi-square test showed statistically significant difference in DcR3 expression between esophagus cancer tissue and adjacent normal tissue ($\chi^2 = 7.84$, $P < 0.01$, **Table 1**).

Both MMP-2 and DcR3 expression in esophageal cancer tissue were correlated with tumor size, lymphatic metastasis, infiltration degree,

Table 1. MMP-2 and DcR3 in esophageal cancer tissue and adjacent normal tissue

Groups	n	MMP-2		DcR3	
		Expressed	Unexpressed	Expressed	Unexpressed
Esophageal cancer tissues	150	81	69	82	68
Adjacent normal tissues	30	5	25	8	22
χ^2		13.97		7.84	
P		< 0.01		< 0.01	

clinical stage, and 3-year survival rate, but not correlated with gender, age, or differentiation degree (**Table 2**). Kendall correlation analysis showed that, MMP-2 and DcR3 were positively correlated in esophageal cancer tissue (r = 0.37, P < 0.01, **Table 3**).

Correlation between expression of MMP-2 and DcR3 in esophageal cancer tissue and post-operative survival

Analysis of Kaplan-Meier survival curves showed that the median survival period of patients with negative MMP-2 expression was 67.0 months, but the median survival period of patients with positive MMP-2 expression was 40.0 months. This indicates that the post-operative survival period significantly decreased with increasing positive expression of MMP-2. A log-rank test revealed a statistically significant difference (χ^2 = 37.29, P < 0.01). The median survival of patients with negative DcR3 expression was 62.0 months, but the median survival of patients with positive DcR3 expression was 44.0. This indicates that post-operative survival significantly decreased with increasing positive expression of DcR3. A log-rank test showed a statistically significant difference (χ^2 = 10.97, P < 0.01).

Discussions

MMP-2, also known as type IV collagenase or gelatinase, is a zinc-dependent proteolytic enzyme secreted by tumor cells and stromal cells as an inactive zymogen that gets activated by hydrolysis [6]. Recently, studies show high MMP-2 expression in esophageal squamous cell carcinoma tissues which is related to esophageal cancer gene expression. MMP-2 may therefore be an early event in esophageal cancer progression [7]. Results from this study showed that the rate of positive MMP-2 expression in esophageal cancer tissue was 54.0%, higher than that in adjacent normal tissues, further indicating that MMP-2 may be involved in occurrence and development of esophageal cancer.

Invasion and metastasis are features of malignant tumors and result from a series of complex and multi-step interactions among tumor cells, host cells and extracellular matrix [8]. Metastasis involves repeated degradation of extracellular matrix and basement membrane. The invasion and metastatic capability of tumor cells is closely related to its ability to produce or induce production of proteases that will degrade extracellular matrix [9]. This study found that MMP-2 positive expression in esophageal cancer tissue is closely associated with tumor size, lymphatic metastasis, infiltration degree, and clinical stage. These findings confirm that MMP-2, as a key enzyme for degrading basement membrane and extracellular matrix, plays an important role in invasion and metastasis of malignant tumors.

DcR3 protein is a recently discovered member of the tumor necrosis factor receptor superfamily; it is a secretory-type soluble protein that is highly expressed in esophageal adenocarcinoma, gastrointestinal cancer, liver cancer, breast cancer, lung cancer, kidney cancer, ovarian cancer, and other tumors. DcR3 protein is closely related to cell differentiation and to tumor occurrence, progression and prognosis [10, 11]. This current study found that the positive rate of DcR3 (54.67%) in esophageal cancer tissues was higher compared to adjacent normal tissues (26.67%), and the positive expression was closely correlated with tumor size, lymphatic metastasis, infiltration degree, and clinical stage. One possible mechanism may be that DcR3 can competitively bind with FasL, LIGHT and T L1A to inhibit apoptosis [12]. Thus, DcR3 protein detection may reveal capacity for malignancy of esophageal cancer that could impact treatment decisions.

This study showed that DcR3 and MMP-2 were positively correlated, consistent with results

Table 2. Correlation between positive expression of MMP-2 and DcR3 and clinicopathologic characteristics of esophageal cancer

Characteristics	n	MMP-2			DcR3		
		Expressed (%)	χ^2	*P*	Expressed (%)	χ^2	*P*
Gender							
Male	96	57 (59.38)	3.1	0.08	55 (57.29)	0.74	0.39
Female	54	24 (44.44)			27 (50.00)		
Age (years)							
< 60	68	32 (47.06)	2.41	0.12	32 (47.06)	2.91	0.09
≥ 60	82	49 (59.76)			50 (60.98)		
Tumor size (cm)							
< 3	41	16 (39.02)	8.00	0.02	21 (51.22)	7.47	0.02
> 3	61	40 (65.57)			47 (77.05)		
> 5	48	22 (45.83)			30 (62.50)		
Differentiation degree							
Excellent	40	23 (57.50)	4.36	0.11	23 (57.50)	3.28	0.19
Moderate	63	42 (66.67)			45 (71.43)		
Poor	47	22 (46.81)			35 (74.47)		
Lymphatic metastasis							
Yes	42	32 (76.19)	9.06	< 0.01	38 (90.48)	22.58	< 0.01
No	108	53 (49.07)			52 (48.15)		
Infiltration degree							
Muscular layer	55	21 (38.18)	14.00	< 0.01	28 (50.91)	8.81	< 0.01
Whole	95	66 (69.47)			71 (74.74)		
Clinical stage							
I/II	77	36 (46.75)	9.26	< 0.01	40 (51.95)	9.98	< 0.01
III/IV	73	52 (71.23)			56 (76.71)		
Survival period							
≥ 3 years	76	35 (46.05)	10.11	< 0.01	36 (47.37)	18.50	< 0.01
< 3 years	74	53 (71.62)			60 (81.08)		

Table 3. Correlation between expressions of MMP-2 and DcR3 in esophageal cancer tissue

MMP-2	DcR3		In total	*r*	*P*
	Expressed	Unexpressed			
Expressed	23	58	81		
Unexpressed	45	24	69		
In total	68	82	150	0.37	< 0.01

reported by Yang *et al*. [13]. The mechanism of this association is not clear. One possible mechanism involves interaction of DcR3 and TL1A to up-regulate expression and activity of MMP-2, thereby promoting tumor angiogenesis and immune system evasion [14]. TL1A may induce generation of extracellular matrix-degrading enzyme through binding with death receptor 3 (DR3), while DcR3 can compete with DR3 to bind with TL1A to limit MMP-2-mediated matrix degradation [15]. MMPs and DcR3 have synergistic effects during inhibition of apoptosis; DcR3 can phosphorylate MMP-2 and change its activity through many signaling molecules including protein kinase A (PKA) and protein kinase C (PKC). Simultaneous detection of MMP and DcR3 expression in tumor tissues is more helpful than detection of one single protein for early diagnosis of esophageal squamous cell carcinoma, judgment of malignancy, estimation of metastatic potential and prognostic evaluation. This information could further guide selection of rational treatment programs.

In addition, the study revealed that post-operative survival rates of patients with negative MMP-2 and negative DcR3 expression were

higher compared to patients with positive MMP-2 and positive DcR3 expression. Log-rank test results showed a statistically significant difference ($P < 0.05$); this was consistent with study results reported by Mrena *et al.* [16] and Macher-Goeppinger *et al.* [17]. A possible mechanism for this effect could include MMP-2 expression promoting extracellular matrix degradation, regulating cell adhesion, and promoting tumor angiogenesis. DcR3 expression could inhibit cell apoptosis and down-regulate host immune function, enabling tumor cells to evade immune surveillance and clearance. Therefore, concurrent reduction in MMP-2 and DcR3 expression could promote esophageal cancer progression.

This study suggests that esophageal tissue MMP-2 and DcR3 expression are important reference values for assisting cancer diagnosis, analyzing disease course, guiding treatment, monitoring recurrence or metastasis, and estimating prognosis. However, the sensitivity and specificity of these two indicators requires further confirmation in a larger and more diverse sample size. Furthermore, in addition to these two biomarkers, protein antigens, hormones, embryonic antigens, and other biomarkers may also have value in diagnosis, treatment and prognosis of esophageal cancer [18]. It is therefore recommended that further studies should compare MMP-2 and DcR3 to other biomarkers and to then select the most cost-effective biomarker combinations for clinical application.

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Ran Ao, Department of Gastroenterology, The First Affiliated Hospital of China Medical University, 155 North Nanjing Street, Shenyang 110001, China. E-mail: aoran6556@126.com

References

[1] Artandi SE, DePinho RA. Telomeres and telomerase in cancer. Carcinogenesis 2010; 31: 9-18.

[2] Yang CR, Hsieh SL, HO FM, Lin WW. Decoy receptor 3 increases monocyte adhesion to endothelial cells via NF-Kappa B-dependent upregulation of intercellular adhesion molecule-1, Vcan-1, and IL-8 expression. J Immunol 2005; 174: 1647-1649.

[3] Yamana K, Bilim V, Hara N. Prognostic Impact of FAS/CD95/APO-1 in urothelial cancers: decreased expression of Fas is associated with disease progression. Br J Cancer 2005; 93: 544-551.

[4] Hsu TL, Wu YY, Chang YC, Yang CY, Lai MZ, Su WB, Hsieh SL. Attenuation of Th1 Response in decoy receptor 3 transgenic mice. Immunol 2005; 175: 5135-5145.

[5] Chen J, Zhang L, Kim S. Quantification and detection of DcR3, a decoy receptor in TNFR family. J Immunol Methods 2004; 285: 63-70.

[6] Yamashita K, Upadhay S, Mimori K, Inoue H, Mori M. Clinical significance of secreted protein acidic and rich in cystein in esophageal carcinoma and its relation to carcinoma progression. Cancer 2003; 97: 2412-2419.

[7] Samantaray S, Sharma R, Chattopadhyaya TK, Gupta SD, Ralhan R. Increased expression of MMP-2 and MMP-9 in esophageal squamous cell carcinoma. Cancer Res Clin Oncol 2004; 130: 37-44.

[8] Yue QF, Xiang M, Li FM. The Expression and Clinical Significance of DcR3 and MMP-2 in Esophageal Squamous Cell Carcinoma. Chin J Clinical Med 2010; 17: 655-658.

[9] Quaranta M, Daniele A, Coviello M, Venneri MT, Abbate I, Caringella ME, Di Tardo S, Divella R, Trerotoli P, Di Gennaro M, Schittulli F, Fransvea E, Giannelli G. MMP-2, MMP-9, VEGF and CA 15.3 in breast cancer. Anticancer Res 2007; 27: 3593-3600.

[10] Hayashi S, Miura Y, Nishiyama T, Mitani M, Tateishi K, Sakai Y, Hashiramoto A, Kurosaka M, Shiozawa S, Doita M. Decoy receptor 3 expressed in rheumatoid synovial fibroblasts protects the cells against fas-induced apoptosis. Arthritis Rheum 2007; 56: 1067-1075.

[11] Zhan C, Yan Q, Patskovsky Y, Li Z, Toro R, Meyer A, Cheng H, Brenowitz M, Nathenson SG, Almo SC. Biochemical and structural characterization of the human TL1A ectodomain. Biochemistry 2009; 48: 7636-7645.

[12] Li WZ, Zhang CG, Chen CX, Zhuang G. Correlation between expression of DcR3 on tumor cells and sensitivity to FasL. Cell Mol Immunol 2007; 4: 455-460.

[13] Yang CR, Hsieh SL, Teng CM, Ho FM, Su WL, Lin WW. Soluable decoy receptor 3 induces angiogenesis by neutralization of TL1A, a cytokine belonging to tumor necrosis factor superfamily and exhibiting angiostatic action. Cancer Res 2004; 64: 1122-1129.

[14] Connor JP, Felder M. Ascites from epithelial ovarian cancer contain high levels of functional decoy receptor 3 (DcR3) and is associated with platinum resistance. Gynecol Oncol 2008; 111: 330-335.

[15] Chen C, Zhang C, Zhuang G, Luo H, Su J, Yin P, Wang J. Decoy receptor 3 overexpression and immunologic tolerance in hepatocellular carcinoma (HCC) development. Cancer Invest 2008; 26: 965-974.
[16] Mrena J, Wiksten JP, Nordling S, Kokkola A, Ristimäki A, Haglund C. MMP-2 but not MMP-9 associated with COX-2 and survival in gastric cancer. J Clin Pathol 2006; 59: 618-623.
[17] Macher-Goeppinger S, Aulmann S, Wagener N, Funke B, Tagscherer KE, Haferkamp A, Hohenfellner M, Kim S, Autschbach F, Schirmacher P, Roth W. Decoy receptor 3 is a prognostic factor in renal cell cancer. Neoplasia 2008; 10: 1049-1056.
[18] He J, Ding JB, Yu WZ. Diagnostic value of multiple tumor markers protein biochip for patients with esophageal carcinoma. Shanxi Med J 2011; 40: 280-283.