Journal of the Egyptian National Cancer Institute (2015) 27, 1–9

Cairo University

Journal of the Egyptian National Cancer Institute

www.elsevier.com/locate/jnci
www.sciencedirect.com

Full Length Article

Expression of a disintegrin and metalloprotease 8 and endostatin in human osteosarcoma: Implication in tumor progression and prognosis

Dalia M. Abd El-Rehim *, Nisreen A.A. Osman

Pathology Department, Faculty of Medicine, Minia University, Egypt

Received 10 September 2014; revised 2 November 2014; accepted 3 November 2014
Available online 4 December 2014

KEYWORDS

Osteosarcoma;
ADAM8;
Endostatin;
Immunohistochemistry

Abstract *Background:* A disintegrin and metalloprotease 8 (ADAM8) is a trans-membrane protein, which is involved in cell adhesion, signaling and migration as well as the proteolytic cleavage of various substrates. Endostatin is a potent inhibitor of angiogenesis. ADAM8 and Endostatin have been associated with multiple malignancies. However, their role in osteosarcoma is not fully elucidated.

Aim: To determine the expression of ADAM8 and endostatin in osteosarcoma and to study their correlation with different clinicopathological parameters and patients' outcomes.

Material and methods: ADAM8 and endostatin expression were immunohistochemically evaluated in 61 primary osteosarcomas and 11 pulmonary metastatic osteosarcoma lesions.

Results: Among 61 primary osteosarcomas, ADAM8 was detected in 52 tumors (85.2%) and highly expressed in 33 cases (54.1%). Positive endostatin expression was found in 28 tumors (45.9%). Higher ADAM8 and decreased endostatin expression rates in metastatic lesions compared to primary osteosarcoma were found but these differences were not statistically significant ($p = 0.086$ & 0.558 respectively). High ADAM8 expression score and positive endostatin expression were significantly correlated with tumor size, stage and distant metastasis ($p < 0.05$). Survival analysis showed that high ADAM8 expression was associated with poor overall survival (OS) ($p = 0.0002$). Multivariate analysis revealed that ADAM8 expression level was an independent prognostic parameter for the OS ($p = 0.017$).

Conclusion: Our data suggest that ADAM8 and endostatin play a role in osteosarcoma progression. High ADAM8 expression serves as a reliable marker for poor prognosis in osteosarcoma patients.

Introduction

Osteosarcoma (OS) is the most common, nonhematopoietic, primary bone malignancy particularly among children and adolescents with a second incidence peak in the elderly [1].

* Corresponding author at: Department of Pathology, Faculty of Medicine, Minia University, 61111, Egypt. Tel.: +20 1007845348.
E-mail address: dalia_abdelrehim@hotmail.com (D.M. Abd El-Rehim).
Peer review under responsibility of The National Cancer Institute, Cairo University.

http://dx.doi.org/10.1016/j.jnci.2014.11.001

In Egypt, it ranks the first, constituting 47.75% among primary malignant bone tumors [2].

Osteosarcoma is a devastating tumor, characterized by high local aggressiveness, rapid growth rate and early metastasis to lungs and distant bones [3]. The ability of osteosarcoma cells to invade and metastasize depends on dynamic changes including cell–cell and cell–matrix interactions. The extracellular matrix degradation is an essential process for tumor cell invasion and angiogenesis [3]. Tumor angiogenesis, in turn, is crucial for sustained osteosarcoma growth and dissemination [3,4].

Despite significant improvements made over the past decades in the therapeutic approach of osteosarcoma, patients with metastases still have a much worse outcome [5]. A better understanding of the mechanisms contributing to the progression of osteosarcoma provides novel opportunities for the effective diagnosis, prognosis, and tumor-targeted therapies.

A disintegrin and metalloprotease (ADAM) family is a group of multidomain proteins consisting of pro-peptide, metalloprotease, disintegrin-like, cysteine rich, EGF-like, transmembrane and cytoplasmic domains. ADAM8 (CD156) is a transmembrane protein that belongs to ADAM family. Within its metalloproteinase domain, ADAM8 possesses the HEXXXHXXGXXH motif required for proteolytic activity while the disintegrin domain is involved in binding to integrins, thereby promoting cellular interactions. It is a multifunctional protein involved in many biological functions including cell adhesion, signaling and migration. It is also, involved in the proteolytic cleavage of various substrates such as cytokine receptors, their ligands, cell adhesion molecules and extracellular matrix components [6,7].

ADAM8 is expressed primarily on immune cells and can be induced with various pro-inflammatory stimuli [8]. Its expression was detected under several pathological conditions characterized by inflammation and extracellular matrix remodeling, including cancer [7]. Upregulation of ADAM8 has been reported in different types of cancers including prostate, breast, hepatocellular carcinomas and pediatric medulloblastoma [9–12].

Endostatin, a recently discovered potent anti-angiogenic factor, generated from the proteolytic cleavage of collagen XVIII that is localized in the vascular and epithelial basement membranes of various organs. Proteolytic degradation of the subendothelial basement membrane releases antiangiogenic fragments that oppose the neoangiogenesis in areas of induced angiogenesis via a negative effect on endothelial cell proliferation and migration [13,14].

Despite its potent antiangiogenic role, elevated levels of serum and/or tissue endostatin have been reported to be associated with aggressive features and poor prognosis in several tumors [15–18] making studying of endostatin expression a subject of interest for several investigators.

Unlike chemotherapeutics, endostatin has no toxicity effect because it acts on the endothelial cells that line blood vessels without harming other cells. Moreover, cancers do not become resistant to endostatin, because endothelial cells divide slowly, making them unlikely to acquire mutations that confer drug resistance [19,20]. Furthermore, antiangiogenic therapy using endostatin has the potential to prevent post-operative progression of pulmonary metastasis from osteosarcoma [21].

The expression pattern of ADAM8 and endostatin and their role in osteosarcoma progression is still unclear. The present work studied the immunohistochemical expression of ADAM8 and endostatin in primary osteosarcoma and pulmonary metastatic osteosarcoma lesions and further investigated their clinicopathological and the prognostic values in this tumor.

Materials and methods

Patients and tissue samples

Sixty-one primary osteosarcoma cases were collected from Pathology Department, Minia University Hospital and Minia Oncology Center, El-Minia, Egypt, during the period from 2009 to 2013. The corresponding clinical information and follow up data were obtained from patients' medical records and presented in Table 1. Of 61 patients, there were 38 male and 23 female patients with a mean age of 24.59 ± 17.18 years and median 18 years (range, 9–69 years). Fifty-four cases had osteosarcoma in the extremities and 7 cases had osteosarcoma in axial skeleton. The resected specimens were reviewed histologically to confirm pathological diagnosis. Staging, using Musculoskeletal Tumor Society (Enneking) staging system for malignant bone lesions, was determined according to established criteria [22]. Overall survival was calculated in months from the date of diagnosis and ended with the time of the tumor-related death or the last follow-up visit of the patient. Another group of 11 excised metastatic pulmonary lesions

Table 1 Clinicopathological features of 61 primary osteosarcoma cases.

Clinicopathological features	*n* (%)
Age	
⩽18 years	33 (54.1%)
> 18 years	28 (45.9%)
Gender	
Male	38 (62.3%)
Female	23 (37.7%)
Tumor site	
Extremities	54 (88.5%)
Axial	7 (11.5%)
Tumor type	
Osteoblastic	39 (63.9%)
Chondroblastic	17 (27.9%)
Fibroblastic	7 (8.2%)
Tumor size	
⩽8 cm	24 (39.3%)
> 8 cm	37 (60.7%)
Stage	
IA	6 (9.8%)
IB	7 (11.5%)
IIA	10 (16.4%)
IIB	26 (42.6%)
III	12 (19.7%)
Metastasis at diagnosis	
No	49 (80.3%)
Yes	12 (19.7%)

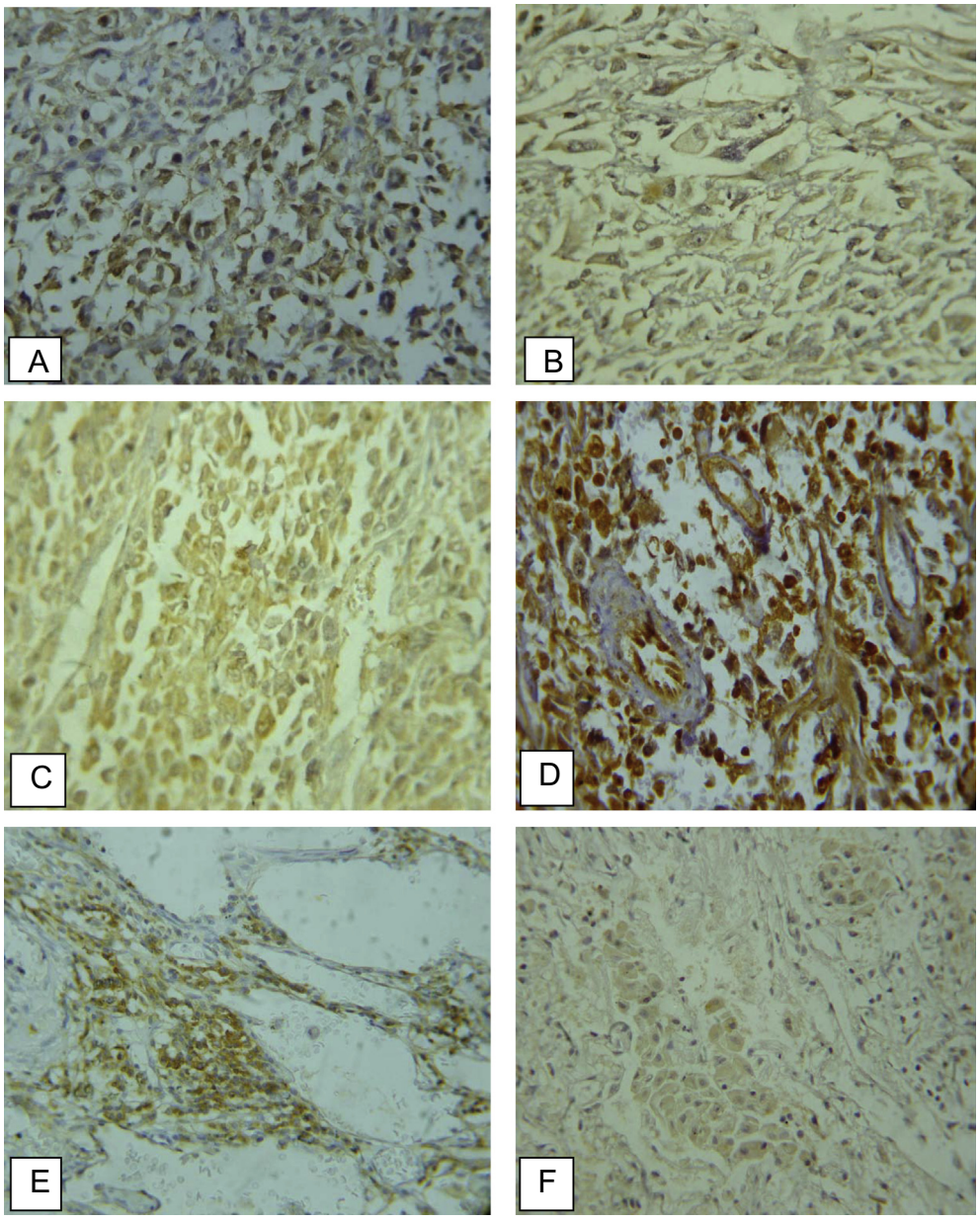

Figure 1 (A)–(F): Immunohistochemical expression of ADAM8 and endostatin in primary and metastatic osteosarcoma. (A): High expression score of ADAM8 in primary osteosarcoma. (B): Low expression score of ADAM8 in primary osteosarcoma. (C): Positive endostatin expression in primary osteosarcoma. (D): Positive endostatin expression in tumor associated blood vessels. (E): High ADAM8 expression in metastatic lesions. (F): Positive endostatin expression in metastatic lesions. Immunohistochemistry: Diaminobenzidine (DAB) chromogen and Mayer's hematoxylin counterstaining. Original magnifications are 400×.

was also included in the present work. Immunostaining was performed on archived paraffin wax embedded specimens.

Immunohistochemical analysis

Immunohistochemistry was performed on sections of 5 μm thickness from each paraffin-embedded specimen. The sections were dewaxed in xylene and rehydrated at graded alcohol. Endogenous peroxide was blocked with 0.3% hydrogen peroxide in methanol for 30 min at room temperature. For antigen retrieval, sections were pretreated in a microwave oven in citrate buffer solution (pH 6) for 10 min and then left at room temperature for 20 min. The slides were then incubated at 4 °C overnight with the primary antibodies: monoclonal mouse anti-human endostatin antibody (1837-46), diluted at 1:100, ThermoFisher Scientific and monoclonal mouse ADAM8 antibody (MAA620Hu22), diluted at 1:100, Life-Science Inc. Secondary antibody for 30 min was used, followed by color detection using a DAB kit. Sections were counterstained with Mayer's hematoxylin, dehydrated in ethanol,

cleared in xylene and mounted. A negative control was generated by using phosphate buffered solution (PBS) to replace the primary antibody. Sections of human placenta and kidney tissues were used as the positive control for endostatin and ADAM8 respectively.

Scoring of immunostaining

Immunostaining was scored by two independent pathologists, who were blinded to the clinicopathological parameters and clinical outcomes of the patients. The scores of the two pathologists were compared and any discrepant scores were trained through re-examining the staining by both pathologists to achieve a consensus score.

The expression pattern of ADAM8 was heterogenous. Therefore, the immunoreactivity score for ADAM8 was based on two parameters: the proportion of positive cells and the intensity of immunoreactivity according to previous studies [10,23]. The number of positive-staining cells showing immunoreactivity in ten representative random microscopic fields was counted and the percentage of positive cells was calculated. The frequency of ADAM8 immunoreactivity in tissue sections was evaluated as '0' when no positive cells were observed within the tumor, '1' when $<25\%$ of the tumor cells were positive, '2' when 25% to 50% of the tumor cells were positive, '3' when 50% to 75% of tumor cells were positive and '4' when $>75\%$ of tumor cells were positive. The intensity of staining was evaluated as 0, 1, 2, and 3 for no staining, weak staining, moderate staining, and strong staining, respectively. A total score of 0–12 was finally calculated by multiplying the percentage and the intensity score. The median value of the total score was calculated. ADAM8 protein expression levels were further classified as low (total score value less than median level) and high (total score value equal or greater than median level). Endostatin expression was evaluated according to a previous work [24]. Cases were considered positive when $\geqslant 10\%$ of tumor cells showed positive expression.

Statistical analysis

The software of SPSS version 11.0 for Windows (SPSS Inc., IL, USA) was used for statistical analysis. The Chi-square test was used to show differences of categorical variables. Patient survival and their differences were determined by Kaplan–Meier method and log-rank test. Cox regression was carried out for multivariate analysis to assess the specific impact of each variable on survival in the presence of other variables. Only variables of significant value from the univariate analysis were entered into the Cox regression analysis. A p-value $\leqslant 0.05$ was considered statistically significant.

Results

ADAM8 and endostatin expression and their correlations with clinicopathological features in primary osteosarcoma

ADAM8 and endostatin expression were analyzed in osteosarcoma tissues ($n = 61$) and in lung metastatic osteosarcoma lesions ($n = 11$). ADAM8 expression was mainly localized to the cytoplasm and plasma membrane of malignant cells. The total ADAM8 expression score range was 0–12 with a median score was 6. Among 61 osteosarcoma specimens, positive ADAM8 immunoreactivity was detected in 52 samples (85.2%) and highly expressed in 33/61 (54.1%) of tumors (Fig. 1A, B). The statistical analyses revealed significant positive associations between high ADAM8 expression and tumor size, stage and metastasis ($p = 0.036$, 0.013, 0.023 respectively). No significant associations were observed between ADAM8 expression and patients' age, gender, tumor site or histological subtype (Table 2).

Positive cytoplasmic endostatin expression was detected in 45.9% (28/61) of Primary tumors. Positive endostatin expression in tumor associated blood vessels was also found (Fig. 1C, D). The expression of endostatin was significantly correlated with tumor size, tumor stage and the presence of distant metastases ($p = 0.035$, 0.012, 0.024 respectively). In contrast, no significant correlation was detected between endostatin expression and other clinicopathological features (Table 2).

ADAM8 and endostatin expression in pulmonary metastatic lesions

All pulmonary metastatic osteosarcoma were ADAM8 positive. Nine cases out of eleven (81.8%) had high ADAM8 expression (Fig. 1E) while 2/11 (18.2%) had low expression. Concerning endostatin expression, 4/11 (36.4%) of metastatic lesions were positive (Fig. 1F). As shown in Table 3, no significant differences were detected between the primary osteosarcoma cases and the pulmonary metastatic lesions regarding ADAM8 and endostatin expression ($p = 0.086$ & 0.558 respectively). However, a trend toward a higher ADAM8 expression in pulmonary metastatic lesions compared to primary tumors was observed.

The relationship between ADAM8 and endostatin expression in primary and pulmonary metastatic osteosarcoma

A significant positive association was found between ADAM8 and endostatin expression ($p = 0.012$) in primary osteosarcoma cases, where a significant proportion of endostatin positive tumors (71.4%) showed high ADAM8 expression scores. In pulmonary metastatic lesions, no significant association was observed between both markers ($p = 0.237$) (Table 4).

Prognostic values of ADAM8 and endostatin expression in osteosarcoma

The median overall survival period was 25 months (range 10–60 months). The prognostic values of ADAM8 and endostatin together with other clinicopathological parameters were evaluated (Table 5). Osteosarcoma cases with high ADAM8 expression were significantly related with shorter overall survival ($p = 0.0002$) (Fig. 2A). The median OS time for osteosarcoma patients with high ADAM8 expression was 24.2 months compared to 60 months in patients with low ADAM8 expression. Regarding endostatin expression, no significant relation between the expression of endostatin and OS was found ($p = 0.133$) (Fig. 2B). Moreover, the survival benefits were found in patients with smaller tumor size ($p = 0.037$), lower

Table 2 Associations of ADAM8 and Endostatin expression with clinicopathological features in primary osteosarcoma.

Clinicopathological features	*N*	ADAM8 expression score			Endostatin expression		
		Low	High	*p*-Value	−ve	+ve	*p*-Value
Age							
⩽18 years	33	17 (51.5%)	16 (48.5%)	0.340	20 (60.6%)	13 (39.4%)	0.268
>18 years	28	11 (39.3%)	17 (60.7%)		13 (46.4%)	15 (53.6%)	
Gender							
Male	38	15 (39.5%)	23 (60.5%)	0.195	19 (50%)	19 (50%)	0.409
Female	23	13 (56.5%)	10 (43.5%)		14 (60.9%)	9 (39.1%)	
Tumor site							
Extremities	54	25 (46.3%)	29 (53.7%)	0.864	28 (51.9%)	26 (48.1%)	0.328
Axial	7	3 (42.9%)	4 (59.1%)		5 (71.4%)	2 (28.6%)	
Tumor type							
Osteoblastic	39	16 (41%)	23 (59%)	0.256	21 (53.8%)	18 (46.2%)	0.429
Chondroblastic	17	8 (47.1%)	9 (52.9%)		8 (47.1%)	9 (52.9%)	
Fibroblastic	5	4 (80%)	1 (20%)		4 (80%)	1 (20%)	
Tumor size							
⩽8 cm	24	15 (62.5%)	9 (37.5%)	0.036	17 (70.8%)	7 (29.2%)	0.0.035
>8 cm	37	13 (35.1%)	24 (64.9%)		16 (43.2%)	21 (56.8%)	
Stage							
IA	6	5 (83.3%)	1 (16.7%)	0.013	5 (83.3%)	1 (16.7%)	0.012
IB	7	6 (85.7%)	1 (14.3%)		7 (100%)	0	
IIA	10	5 (50%)	5 (50%)		6 (60%)	4 (40%)	
IIB	26	10 (38.5%)	16 (61.5%)		12 (46.2%)	14 (53.8%)	
III	12	2 (16.7%)	10 (83.3%)		3 (25%)	9 (75%)	
Metastasis at diagnosis							
No	49	26 (53.1%)	23 (46.9%)	0.023	30 (61.2%)	19 (38.8%)	0.024
Yes	12	2 (16.7%)	10 (83.3)		3 (25%)	9 (75%)	

Test of significance: Chi-square test and Fisher's exact test. *p*-value ⩽0.05 is considered significant.

Table 3 Comparison between primary and pulmonary metastatic osteosarcoma cases regarding ADAM8 and Endostatin expression.

Variable	Lesions		*p*-Value
	Primary osteosarcoma ($n = 61$)	Pulmonary metastatic lesion ($n = 11$)	
ADAM8 expression score			
Low	28 (45.9%)	2 (18.2%)	0.086
High	33 (54.1%)	9 (81.8%)	
Endostatin expression			
Negative	33 (54.1%)	7 (63.6%)	0.558
Positive	28 (45.9%)	4 (36.4%)	

Test of significance: Chi-square test and Fisher's exact test. *p*-Value ⩽0.05 is considered significant.

clinical stage ($p < 0.0001$) and without distant metastasis ($p < 0.0001$) for OS.

Multivariate Cox regression analysis, enrolling the above mentioned significant parameters, demonstrated the value of ADAM8 expression for predicting OS of osteosarcoma patients. High ADAM8 expression and the presence of metastasis at diagnosis were identified as the independent poor prognostic factors for OS ($p = 0.017$ and 0.003 respectively) of patients with osteosarcoma. ADAM8 expression displayed a powerful efficacy in predicting outcome, where it had comparable hazard ratio (HR) with metastasis at diagnosis in refining OS. High ADAM8 expression score was shown to adversely influence OS with a HR of 4.549 (95% CI 1.313–15.759), representing an increase in the risk of dying of osteosarcoma patients (Table 6).

Discussion

Accumulating evidence has demonstrated ADAM8 overexpression in a variety of human tumors [9–12,23]. The current study identified abundant ADAM8 expression in osteosarcoma and osteosarcoma derived lung metastases. Herein, positive ADAM8 expression rate was 85.2% in primary osteosarcoma. High ADAM8 expression scores were observed in 54.1% of tumors. This finding was in agreement with a previous study, which found that 88.4% of

Table 4 Association between ADAM8 and Endostatin expression in primary osteosarcoma and pulmonary metastatic osteosarcoma.

	ADAM8 expression score		χ^2	Phi	*p*-Value
Endostatin expression	Low	High			
Primary osteosarcoma n = 61					
Negative	20 (60.6%)	13 (39.4%)	6.260	0.320	0.012
Positive	8 (28.6%)	20 (71.4%)			
Pulmonary metastatic osteosarcoma n = 11					
Negative	2 (28.6%)	5 (71.4%)	1.397	0.356	0.237
Positive	–	4 (100%)			

Test of significance: Chi-square test and Fisher's exact test. *p*-Value ⩽0.05 is considered significant. High association: Phi >0.5; moderate association: Phi = 0.3–0.5; low association: Phi = 0.1–0.3; little if any association: Phi = 0–0.1.

Table 5 Univariate analyses for OS in 61 patients with osteosarcoma.

Item variables	Overall survival			*p*-Value
	Median OS	12 month rate % (SE)	36 month rate % (SE)	
Age				
⩽18 years	54.2	76 (0.61)	57 (0.80)	0.455
>18 years	35.8	64 (0.92)	50 (0.84)	
Gender				
Male	43.4	67 (0.68)	46 (0.69)	0.685
Female	54.9	77 (0.82)	62 (0.87)	
Tumor Site				
Extremities	48.4	67 (0.49)	50 (0.66)	0.184
Axial	36.0	100 (1.41)	75 (1.40)	
Tumor type				
Osteoblastic	46.3	69 (0.70)	49 (0.37)	0.666
Chondroblastic	48.7	72 (0.79)	52 (0.71)	
Fibroblastic	48.0	80 (1.07)	80 (1.48)	
Tumor size				
⩽8 cm	60.0	86 (1.31)	72 (1.27)	0.037
>8 Cm	35.9	60 (0.44)	40 (0.53)	
Stage				
IA	60.0	82 (1.10)	82 (1.52)	<0.0001
IB	48.0	100 (1.41)	78 (1.47)	
IIA	57.0	100 (1.41)	80 (1.52)	
IIB	47.1	79 (0.85)	49 (0.74)	
III	18.1	9 (0.09)	–*	
Metastasis at diagnosis				
No	60.0	86 (0.93)	64 (0.97)	<0.0001
Yes	18.1	9 (0.9)	–*	
ADAM8				
Low	60.0	96 (1.34)	76 (1.48)	0.0002
High	24.2	50 (0.36)	34 (0.34)	
Endostatin				
−ve	58.0	80 (0.85)	66 (0.85)	0.133
+ve	36.6	59 (0.60)	34 (0.54)	

Log rank test was used; *p*-value ⩽0.05 is considered significant; OS: overall survival, DFS: disease free survival, SE: standard error.
* The maximum follow up time was less than 36 months.

osteosarcoma cases were positive for ADAM8 staining and 59.4% had high expression scores [23]. In pulmonary metastatic osteosarcoma lesions, this study noticed higher ADAM8 expression scores compared to primary tumors, indicating ADAM8 up-regulation during tumourigenesis and progression of this tumor.

The present work also, demonstrated significant associations between high ADAM8 expression and large tumor size, advanced stage as well as metastases. Moreover, a statistically significant relation between high ADAM8 expression and unfavorable OS by univariate analysis was found. Multivariate analysis confirmed that high ADAM8 expression was an independent predictor for poor OS. Recently, the association of ADAM8 up-regulation with aggressive tumor features as well as poor patients' prognosis has also been determined by several studies [9–12,23].

ADAM8 is a crucial player in tumorigenesis and metastatic spread of malignant tumors. It facilitates the release of

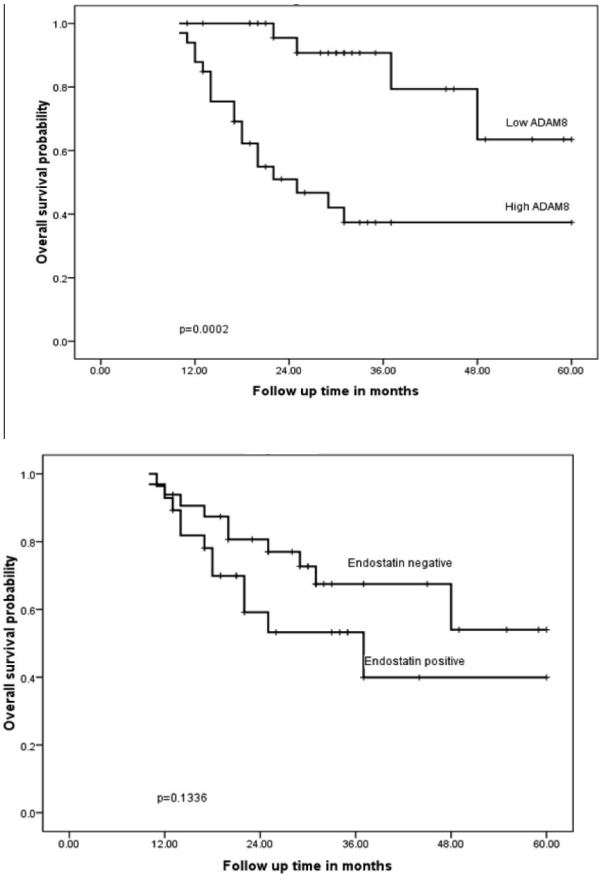

Figure 2 (A)–(D): Kaplan Meier analysis of overall survival in osteosarcoma patients according to ADAM8 (A) and endostatin (B) expression in osteosarcoma tissue samples.

pro-angiogenic factors into the tumor microenvironment, inducing angiogenesis and continued tumor growth. It also activates β1-integrin on the tumor cells permitting their attachment to the vascular endothelium and entry into the circulation thus, increasing the numbers of circulating tumor cells and the risk of developing metastases [12].

Tumor progression and metastasis, in large part, depend on angiogenesis. Several inhibitors of angiogenesis have been identified. Endostatin represents one of the most potent negative regulators of angiogenesis, which exhibits specific inhibitory action on the proliferation, migration, and tube formation of endothelial cells [14].

The generation of endostatin by several human tumors has been reported in the literature [4,16,24–26]. In this study, positive cytoplasmic endostatin expression was detected in 45.9% of tumors, which was consistent with previous studies demonstrating cytoplasmic endostatin expression in the tumor cells of osteosarcoma, breast, hepatocellular and oral squamous cell carcinomas [4,16,24–26], suggesting that the source of endostatin may be partially derived from these types of tumor cells. However, our results were inconsistent with a previous study [15] that reported restricted expression of endostatin to tumor associated blood vessels with no expression by tumor cells of malignant gliomas. Another study [18] demonstrated stage dependent localization of endostatin in bladder carcinoma, being localized to tumor associated blood vessels in superficial carcinoma while strongly expressed in cancer cells in muscle-invasive bladder carcinoma. These discrepancies could be attributed to several factors such as tumor histology, stage and primary site of the tumor. In addition, differences in laboratory detection methods as well as variations in immunoreactivity to different endostatin antibodies used in different studies could be responsible for these variations.

In pulmonary metastatic osteosarcoma lesions, the present study demonstrated that endostatin expression rates were relatively reduced compared to primary tumors however; endostatin expression rates did not differ significantly between primary osteosarcoma and pulmonary metastatic lesions. In agreement with our finding, a previous study demonstrated no significant difference in endostatin immunoreactivities between primary oral squamous cell carcinomas and metastatic nodes; although a trend for diminished endostatin expression in the metastatic nodes was observed [26]. Whether primary tumors may generate more endostatin than metastatic tumors, an issue needs further elucidation on a larger scale of primary and metastatic tumors.

As endostatin is a potent anti-angiogenetic factor, one would expect decreased endostatin levels during tumor progression. Interestingly, the present study demonstrated significant associations between the expression of endostatin and increased tumor size, stage and presence of metastasis. These findings are in agreement with previous studies in osteosarcoma [24], hepatocellular carcinoma [16] and gliomas [15]. Similarly, other studies reported an association between increased serum endostatin levels and aggressive tumor features as well as unfavorable prognosis [17,18,27,28].

However, our findings are inconsistent with two studies that observed a significant association between endostatin expression and decreased metastatic potential in oral squamous cell carcinoma [26] and osteosarcoma [4] cases. However, the latter reported a significant association between positive endostatin expression and poor differentiation in osteosarcoma.

Recent studies have provided reasonable evidence that explain why the anti-angiogenic factor, endostatin, is associated with aggressive features in malignant tumors. During the process of the angiogenesis, large amounts of angiogenic factors are secreted from malignant cells to promote angiogenic phenotype of malignant tumors. The increased levels of

Table 6 Multivariate analyses for OS in 61 patients with osteosarcoma.

Variables	B	SE	*p*-Value	HR (95% CI)
ADAM8	1.515	0.634	0.017	4.549 (1.313–15.759)
Metastasis at diagnosis	2.471	0.821	0.003	11.836 (2.370–59.104)
Tumor size	0.33	0.718	0.964	1.033 (0.253–4.218)
Stage	0.029	0.457	0.950	1.029 (0.420–2.521)

B: regression coefficient, SE: standard error, HR: hazard ratio, CI: confidence interval; *p*-value $\leqslant$0.05 is considered significant.

negative angiogenic factors including endostatin represent a compensatory response to regulate the angiogenesis balance [4,29]. Elevated levels of endostatin in aggressive tumors are also, related to the proteolytic cleavage of endostatin precursor, collagen XVIII, mediated by elevated matrix metalloproteinases (MMPs) family members in these tumors [18,30]. Given the complexity of endostatin generation and function, future studies are warranted to define the mechanisms underlying endostatin up-regulation and function during osteosarcoma progression.

On studying the association of ADAM8 and endostatin expression, the current study identified significant association between both markers in primary osteosarcoma cases where a great proportion of endostatin positive tumors showed high ADAM8 expression scores. To the best of our knowledge, the direct relation between ADAM8 and endostatin has not been discussed in previous literature. Various proteases such as MMP family members have been implicated in endostatin generation from its precursor collagen type XVIII [30]. As the process of angiogenesis is accompanied by intensive remodeling of the extracellular matrix, the degradative action of various proteases likely leads to increased generation of endostatin from collagen type XVIII, one of the constituents of basement membranes as a local control mechanism for the regulation of angiogenesis [18,30,31]. These reports strongly suggest that elevated endostatin level is related to the proteolytic cleavage of collagen XVIII by proteases. ADAM8 was reported to have proteinase activity as its metalloproteinase sequence with the catalytic metalloproteinase domain of MMPs family [6]. ADAM8 expression was also, detected under pathological conditions characterized by extracellular matrix remodeling such as cancer [7], suggesting that its metalloproteinase activity may partially participate in the increased generation of endostatin from collagen type XVIII during cancer progression. In support of our interpretation, high serum endostatin level was significantly correlated with elevated MMP-7 concentrations in bladder cancer [18]. However, the association between ADAM8 and endostatin expression was not exclusive in this series. We also noticed that 28.6% of endostatin positive tumors were either ADAM8 negative or had low ADAM8 expression scores, indicating that the involvement of other proteases in endostatin production should be considered.

In conclusion, the current work showed that ADAM8 was upregulated in primary osteosarcoma tissues and in metastatic pulmonary lesions. Its overexpression was associated with aggressive malignant features. Positive endostatin expression was also, correlated with aggressive tumor features, indicating the role of ADAM8 and endostatin in osteosarcoma progression. Our findings also, suggest ADAM8 as a good marker for advanced and metastatic osteosarcoma. More importantly, ADAM8 expression status was an independent prognostic indicator for predicting the OS in osteosarcoma patients. The relation between ADAM8 and endostatin and the role of various proteases in generation of endostatin need further investigation on a larger scale of tumors.

Conflict of interest

None.

References

[1] Mirabello L, Troisi RJ, Savage SA. Osteosarcoma incidence and survival rates from 1973 to 2004: data from the surveillance, epidemiology, and end results program. Cancer 2009;115(7):1531–43.

[2] Mokhtar N, Gouda I, Adel I, editors. Malignant bone tumors. Cancer pathology registry 2003–2004 and timed trend analysis, 11. Elsheraa Press; 2007. p. 88–9.

[3] Broadhead ML, Clark JC, Myers DE, Dass CR, Choong PF. The molecular pathogenesis of osteosarcoma: a review. Sarcoma 2011. http://dx.doi.org/10.1155/2011/959248, 2011959248.

[4] Xu CJ, Song JF, Su YX, Liu XL. Expression of b-FGF and endostatin and their clinical significance in human osteosarcoma. Orthop Surg 2010;2(4):291–8.

[5] Aljubran AH, Griffin A, Pintilie M, Blackstein M. Osteosarcoma in adolescents and adults: survival analysis with and without lung metastases. Ann Oncol 2009;20:1136–41.

[6] Mochizuki S, Okada Y. ADAMs in cancer cell proliferation and progression. Cancer Sci 2007;98(5):621–8.

[7] Koller G, Schlomann U, Golfi P, Ferdous T, Naus S, Bartsch JW. ADAM8/ MS2/CD156, an emerging drug target in the treatment of inflammatory and invasive pathologies. Curr Pharm Des 2009;15:2272–81.

[8] Hall T, Pegg LE, Pauley AM, Fischer HD, Tomasselli AG, Zack MD. ADAM8 substrate specificity: influence of pH on preprocessing and proteoglycan degradation. Arch Biochem Biophys 2009;491:106–11.

[9] Fritzsche FR, Jung M, Xu C, Rabien A, Schicktanz H, Stephan C, et al. ADAM8 expression in prostate cancer is associated with parameters of unfavorable prognosis. Virchows Arch 2006;449(6):628–36.

[10] Zhang R, Yuan Y, Zuo J, Liu W. Prognostic and clinical implications of a disintegrin and metalloprotease8 expression in pediatric medulloblastoma. J Neurol Sci 2012;323:46–51.

[11] Zhang Y, Tan YF, Jiang C, Zhang K, Zha TZ, Zhang M. High ADAM8 expression is associated with poor prognosis in patients with hepatocellular carcinoma. Pathol Oncol Res 2013;19(1):79–88.

[12] Romagnoli M, Mineva ND, Polmear M, Conrad C, Srinivasan S, Loussouarn D, et al. ADAM8 expression in invasive breast cancer promotes tumor dissemination and metastasis. EMBO Mol Med 2014;2(6):278–94.

[13] Marneros AG, She H, Zambarakji H, Hashizume H, Connolly EJ, Kim I, et al. Endogenous endostatin inhibits choroidal neovascularization. FASEB J 2007;21:3809–18.

[14] Zhuo W, Chen Y, Song X, Luo Y. Endostatin specifically targets both tumor blood vessels and lymphatic vessels. Front Med 2011;5(4):336–40.

[15] Morimoto T, Aoyagi M, Tamaki M, Yoshino Y, Hori H, Duan L, et al. Increased levels of tissue endostatin in human malignant gliomas. Clin Cancer Res 2002;8(9):2933–8.

[16] Hu TH, Huang CC, Wu CL, Lin PR, Liu SY, Lin JW, et al. Increased endostatin/collagen XVIII expression correlates with elevated VEGF level and poor prognosis in hepatocellular carcinoma. Mod Pathol 2005;18(5):663–72.

[17] Kurschat P, Eming S, Nashan D, Krieg T, Mauch C. Early increase in serum levels of the angiogenesis-inhibitor endostatin and of basic fibroblast growth factor in melanoma patients during disease progression. Br J Dermatol 2007;156(4):653–8.

[18] Szarvas T, Vom Dorp F, Reis H, Szendröi A, Romics I. Serum endostatin level correlates with enhanced extracellular matrix degradation and poor patients' prognosis in bladder cancer. Int J Cancer 2012;130(12):2922–99.

[19] Eder JP, Supko JG, Clark TW, Puchalski TA, Garcia-Carbonero R, Ryan DP, et al. Phase I clinical trial of recombinant human endostatin administered as a short

intravenous infusion repeated daily. J Clin Oncol 2002;20(18):3758–60.
[20] Kulke MH, Bergsland EK, Ryan DP, Enzinger PC, Lynch TJ, Zhu AX, et al. Phase II study of recombinant human endostatin in patients with advanced neuroendocrine tumors. J Clin Oncol 2006 Aug1;24(22):3555–61.
[21] Kaya M, Wada T, Nagoya S, Yamashita T. Prevention of postoperative progression of pulmonary metastases in osteosarcoma by antiangiogenic therapy using endostatin. J Orthop Sci 2007;12(6):562–7.
[22] Fletcher CDM, Bridge JA, Hogendoorn P, Mertens F, editors. WHO classification of tumours, IARC WHO classification of tumours. WHO classification of tumours of soft tissue and bone, vol. 5(2). Lyon, France: IARC Press; 2013.
[23] Li Z, Liao Q, Wu Y, Liao M, Hao Y, Zhang S, Song S, Li B, Zhang YD. Upregulation of a disintegrin and metalloprotease8 influences tumor metastasis and prognosis in patients with osteosarcoma. Pathol Oncol Res 2012;18(3):657–61.
[24] Kim HS, Lim SJ, Park YK. Anti-angiogenic factor endostatin in osteosarcoma. AMPIS 2009;117(10):716–23.
[25] Balasubramanian SP, Cross SS, Globe J, Cox A, Brown NJ, Reed MW. Endostatin gene variation and protein levels in breast cancer susceptibility and severity. BMC Cancer 2007;7:107.
[26] Nikitakis NG, Rivera H, Lopes MA, Siavash H, Reynolds MA, Ord RA, Sauk JJ. Immunohistochemical expression of angiogenesis related markers in oral squamous cell carcinomas with multiple metastatic lymph nodes. Am J Clin Pathol 2003;119:574–86.
[27] Homer JJ, Greenman J, Stafford ND. Circulating angiogenic cytokines as tumor markers and prognostic factors in head and neck squamous cell carcinoma. Clin Otolaryngol Allied Sci 2002;27:32–7.
[28] Chen Z, Chen QX, Hou ZY, Hu J, Cao YG. Clinical predictive value of serum angiogenic factor in patients with osteosarcoma. Asian Pac J Cancer Prev 2012;13:4823–6.
[29] Hu Y, Hu MM, Shi GL, Han Y, Li BL. Imbalance between vascular endothelial growth factor and endostatin correlates with the prognosis of operable non-small cell lung cancer. Eur J Surg Oncol 2014;40(9):1136–42.
[30] Heljasvaara R, Nyberg P, Luostarinen J, Parikka M, Heikkilä P, Rehn M, et al. Generation of biologically active endostatin fragments from human collagen XVIII by distinct matrix metalloproteases. Exp Cell Res 2005;307:292–304.
[31] Dreymueller D, Pruessmeyer J, Groth E, Ludwig A. The role of ADAM-mediated shedding in vascular biology. Eur J Cell Biol 2012;91:472–85.