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RESEARCH ARTICLE

Disintegrin and metalloproteinases (ADAMs) expression in gastroesophageal reflux disease and in esophageal adenocarcinoma

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Abstract

Background   Clinically useful marker molecules for the progression of gastroesophageal reflux disease and Barrett's esophagus (BE) to esophageal adenocarcinoma (EAC) are lacking. Many adenocarcinomas and inflammatory conditions exhibit increased expression of ADAMs, 'a disintegrin and metalloproteinases'.

Methods   We assessed the expression of five ADAMs (9, 10, 12, 17, 19) in three esophageal cell lines (Het-1A, OE19, OE33) by RT-PCR and Western blotting, and in human samples of normal esophagus, esophagitis, BE, Barrett's dysplasia, and EAC by RT-PCR, and in selected samples by immunohistochemistry.

Results   EAC patients showed increased mRNA expression of ADAMs 9, 12, 17 and 19, as compared to controls. At immunohistochemistry, ADAM9 and ADAM10 proteins were increased in EAC. Patient samples also showed increased mRNA expression of ADAM12 in esophagitis, of ADAM9 in BE, and of ADAMs 9, 12 and 19 in Barrett's dysplasia, as compared to controls. Two EAC cell lines showed increased ADAM9 mRNA.

Conclusions   ADAM9 expression is increased in EAC. Its predecessors show increased ADAM9 mRNA expression. The importance of the alterations in ADAM expression for the development of EAC, and their use as marker molecules, warrant further studies.

Keywords   Esophageal adenocarcinoma ·
Gastroesophageal reflux disease · Barrett's esophagus ·
ADAM molecules

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Introduction

Esophageal adenocarcinoma (EAC) is associated with a high overall mortality [1], in spite of the advances in its treatment. The prevention of EAC by treating its recognised predisposing factor, gastroesophageal reflux disease (GERD), or its sequel, Barrett's esophagus (BE), by surgery or medication has not proven effective [2–4]. Thus, the search for clinically useful markers of progression from BE to malignancy has been extensive but unsuccessful [5].

As there is no evidence to date of a single decisive transformation pathway leading to EAC, its development is likely to be multifactorial. A molecule subfamily of the metalloproteinases, a disintegrin and metalloproteinases (ADAMs), has been previously linked to both inflammation [6] and malignancy [7], and, therefore, was chosen to be studied in the inflammatory, metaplastic, dysplastic and carcinoma phases of GERD. ADAMs participate in the shedding of growth factors and in cellular adhesion [8]. Their composition entails them the capacity for multiple functions: they consist of eight domains, including a

metalloproteinase and a disintegrin domain, a transmembrane sequence, and a prodomain, the removal of which activates the metalloproteinase domain [9]. Increased expression of ADAMs has been found in many cancers [7], and has been also linked to cancer progression and aggressiveness [10]. ADAMs are naturally inhibited by the tissue inhibitors of metalloproteinases (TIMPs) [7], and oncologic medication targeting them has been developed [10]. Increased ADAM expression has also been found in inflammatory bowel disease and Helicobacter pylori gastritis [11, 12]. Multiple substrates of ADAMs, including transforming growth factor beta (TGF-β) and E-cadherin, have been shown to have a prognostic impact in EAC, although with no major individual clinical relevance [13]. ADAM mRNA expression has been found to be increased in esophago-gastric adenocarcinomas [14]. While defining the origin of the tumours in gastro-esophageal junction is difficult, it is important because of the probably differing etiology. For gastric adenocarcinoma, Helicobacter pylori infection is a strong risk factor, whereas EAC and its risk factor BE bear an inverse relationship with this infection [15].

We, thus, wanted to explore the importance of ADAMs in the GERD-related esophageal and gastroesophageal junctional (Siewert 1 and 2) cancers during their development process. The expression of five ADAM molecules (ADAMs 9, 10, 12, 17 and 19) was assessed at mRNA level, and partly at the protein level. Their expression at different stages of GERD was compared to that in the distal esophagus of normal controls, as well as to that in the proximal esophagus of the GERD and EAC patients. The expression of ADAMs was also analysed in cell lines of an esophageal and a gastroesophageal junctional adenocarcinoma, and from the normal esophagus. ADAM9 expression was increased in EAC at both the protein and mRNA levels, and ADAM9 mRNA was increased in BE and Barrett's dysplasia.

Materials and methods

The study protocol was approved by the Ethics Committee of the Department of Surgery, Helsinki University Hospital.

Cell lines and cell culture

Cell lines of Barrett's adenocarcinoma (OE33), and of adenocarcinoma of the gastroesophageal junction (OE19), the authenticity of which has been confirmed [16], were used. For comparison, Het-1A, a cell line drawn from normal esophageal tissue transfected with a SV40 large T antigen, was used. OE33 and OE19 were purchased from Health Protection Agency Culture Collections (Salisbury, UK), and Het-1A and the gastric carcinoma cell line NCI-N87 that served for standardization from LGC Promochem (Middlesex, UK). The cell lines were cultured in 37 °C in media as follows: OE33, OE19 and NCI-N87 in RPMI +10 % FBS (fetal bovine serum) (Gibco, Carlsbad, CA), and Het-1A in BEGM™ Bronchial Epithelial Cell Growth Medium (Cambrex Bio Science, East Rutherford, NJ).

Patient characteristics

Samples were randomly collected from patients undergoing upper gastrointestinal endoscopy or esophageal surgery during years 2007–2010, and classified according to the histology (Table 1). Controls were patients with normal esophageal mucosa undergoing endoscopy for suspected upper gastrointestinal pathology. One patient was included in two different study groups, as first biopsied BE with mild dysplasia, and transformed later to EAC during follow-up.

Tissue samples

All patients gave a written, informed consent of their participation. Tissue samples were collected in upper gastrointestinal endoscopies, or at surgery due to esophageal adenocarcinoma or high-grade dysplasia. Two to three samples from three different locations were drawn for each patient from both the distal (approx. 2 cm above the gastroesophageal junction), and the proximal esophagus (at approx. 15 cm above the gastroesophageal junction). One sample was placed in RNAlater (Qiagen, Hilden, Germany) for RT-PCR, and other samples from the same location were fast-frozen in liquid nitrogen, or directly placed in 10 % formalin for immunohistochemistry.

Extraction of RNA and proteins

RNA from the cell lines was isolated using the RNeasy Mini Kit (Qiagen, Germantown, MD) according to the protocol specified in the Kit. From the tissue samples, RNA was extracted by the RNeasy Fibrous Tissue Mini Kit from (Qiagen). Tissues were homogenized in the Thermo Savant FastPrep (Qbiogene, Carsbad, CA). RNA was then isolated according to the Kit protocol, except for the double amount of Buffer RLD and Proteinase K used in tissue homogenization. For all mRNA samples, the DNAse Kit (Qiagen, Hilden, Germany) was used to eliminate DNA.

Proteins from the cell lines were extracted using a buffer containing 150 mmol NaCl, 10 mmol Tris–HCl, Tris-Base, 1 mmol EDTA, 1 mmol EGTA, 1 % TritonX-100 and 0.5 % Np-40 with pH of 7.6. Proteinase inhibitors Roche's Complete block A (1 tbl/50 ml) and PMSF (phenylmethylsulfonyl fluoride) were added to the buffer before use.

Table 1 Patient and clinical characteristics

| | Controls | Esophagitis | | BE[a] | BE[a] with dysplasia | | EAC[b] |
		mild	severe		low-grade	high-grade	
n	26	11	5	20	5	7	20
Age mean (range)	55.8 (32.5–80.4)	56.5 (47.3–66.9)	63.4 (57.9–69.2)	62.3 (47.3–76.0)	63.5 (55.3–72.0)	62.9 (42.7–86.0)	66.3 (42.8–85.3)
Males n (%)	9 (34.6)	7 (63.6)	3 (60.0)	18 (90.0)	4 (80.0)	6 (85.7)	17 (85.0)
Location							
Siewert 1 n (%)							7 (35 %)
Siewert 2 n (%)							13 (65 %)
Neoadjuvant n (%)[c]							5 (25 %)
Differentiation							
Good n (%)							6 (30 %)
Moderate n (%)							7 (35 %)
Poor n (%)							7 (35 %)

[a] *BE* Barrett's esophagus

[b] *EAC* Esophageal adenocarcinoma

[c] Had received preoperative neoadjuvant chemo- and/or radiation therapy

Reverse transcription (RT)-PCR

RNA was reversely transcribed to cDNA using the High Capacity cDNA Archive Kit (Applied Biosystems, Foster City, CA). RT-PCR was performed, and the amount of cDNA was quantified, with ABI Prism 7000 Sequence Detection Software exploiting Taqman technology. Primers were purchased from Applied Biosystems for ADAM9 (Hs00177638_m1), ADAM10 (Hs00153853_m1), ADAM12 (Hs01106104_m1), ADAM17 (Hs00234224_ m1), and ADAM19 (Hs00224960_m1). For the cell lines, RT-PCR for each ADAM was run in triplicate, and for the tissue samples, each was run in duplicate.

We used two methods for standardization of the RT-PCR results: The same dilution batch of the cell line NCI-N87 cDNA was run in every plate to count for plate to plate variations. The expression of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) in cell lines, along with that of beta-actin (ACTB) in the tissue samples, was measured for each specimen and used for correction of the ADAM expression.

Western blotting

To quantify protein content of cell lines, we used the Quant-it kit (Invitrogen, Eugene, OR) with fluorescence plate reader (Wallac 1420, Perkin-Elmer, Turku, Finland). Extracted proteins were separated by SDS-electrophoresis on 10 % polyacrylamide gel, and transferred to a nitrocellulose membrane (Bio-Rad, Hercules, CA). Odyssey blocking buffer (Li-Cor, Lincoln, NE) was used for blocking and antibody dilutions. To detect ADAMs, the membrane was incubated overnight with primary antibodies (Online Resource 1) in dilutions 1.0 μg/ml. After incubating with fluorescent-labelled secondary antibodies (dilution 1:15 000) (Online Resource 1), the signal was measured with Odyssey infrared scanner (Li-Cor) and analysed with Odyssey software (v2.1). The GAPDH expression served for standardisation.

Immunohistochemistry

For immunohistochemistry, 6 controls, 3 patients with severe esophagitis, 3 with non-dysplastic BE, 5 with dysplastic BE, and 7 with esophageal adenocarcinoma were selected as the most representative samples, irrespective of their mRNA expression. Immunohistochemistry was performed on formalin-fixed, paraffin-embedded tissue samples. Antigen retrieval was performed by heating the glasses in 0.01 M Na-citrate buffer, at pH 6 and temperature of 98 °C for 20 min. For blocking, Normal Horse Serum 2.5 % (ImmPRESS™ Reagent Kit Universal Anti-Mouse/rabbit Ig, MP-7500, Vector Laboratories,

Burlingame, CA) was used. Glasses were then incubated with primary antibodies overnight at 4 °C. Antibodies were targeting ADAM9 (AB19024, dilution 1:400, Millipore (Billerica, MA)), ADAM10 (AB936, 1:750, R&D Systems, Minneapolis, MN), ADAM12 (LS-B2756, 1:2000, LifeSpanBioSciences (Seattle, WA)), ADAM17, HPA010738-100 UL, 1:50, Sigma-Aldrich (St.Louis, MO)), and ADAM19 (sc25988, 1:200, Santa Cruz Biotechnology, Dallas, TX). After that, slides were soaked with 0.75 % H_2O_2 for 30 min, and then incubated with peroxidase-conjugated secondary antibodies: ImmPRESS^{TM} Reagent Kit peroxidase, Anti-Goat Ig and Universal Anti-Mouse/Rabbit Ig (Vector Laboratories, Burlingame, CA). DAB (3,3′-Diaminobenzidine)was used for visualizing the peroxidase reaction. The slides were coated with Clarion^{TM} Mounting Medium (C0487-100 ml, Sigma-Aldrich, Inc, St.Louis, MO).

The IHC slides were analysed by two of the researchers (LK and TK), and the cellular localisation of ADAMs was assessed. ADAM expression in each sample was graded from 0 to 3 (0 = no staining, 1 = mild staining, 2 = medium staining, 3 = strong staining) based on the intensity and extensity of the staining.

Statistical analysis

Statistical analysis was conducted with SPSS Statistics 22 (SPSS Inc., Chicago, IL). Results are given as mean and standard error of mean (SEM) range, or median and range. Normality of the distributions was assessed with Kolmogorov–Smirnov test. Parametric tests were used for the normally distributed variables, and non-parametric tests otherwise. The unpaired (patients to controls, cell lines) or paired (intrinsic proximal controls) Student's t test was used for normally distributed variables, whereas the Mann–Whitney U test or the Wilcoxon signed-rank test was used for the non-normal data. To compare the different stages of GERD, ANOVA with Dunnett's test to correct for multiple comparisons were used as parametric tests and the Kruskall-Wallis and Dunn's test (to correct for the multiple comparisons) as non-parametric tests. The Jonckheere-Terpstra test was used to evaluate ADAM expression during the control–esophagitis–BE–BE with dysplasia–adenocarcinoma sequence. p value ≤0.05 was considered statistically significant, and two-tailed tests were used.

Results

ADAM mRNA expression in cell lines

The expression of ADAM9 mRNA was increased in both the Barrett's adenocarcinoma cell line OE33, and the
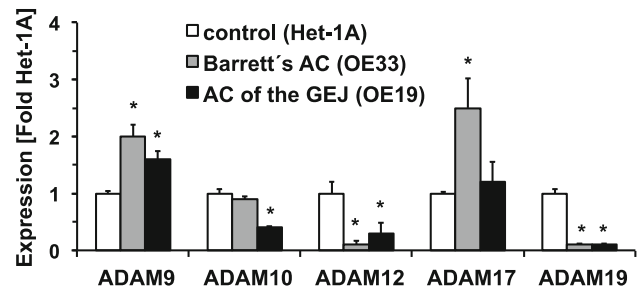

Fig. 1 ADAM mRNA expression in esophageal cell lines relative to expression in Het-1A, and corrected with GAPDH-expression. *Stars* indicate t test comparisons with significant difference ($p \leq 0.05$) between the cell line in question, and Het-1A. *Error bars* represent the standard error of mean. *AC* adenocarcinoma, *GEJ* gastro-esophageal junction

gastro-esophageal junctional adenocarcinoma cell line OE19 as compared to Het-1A (Fig. 1). For ADAM10, its mRNA expression did not differ in OE33, but decreased in OE19. The mRNA expressions of ADAM12 and ADAM19 were decreased in both OE33 and OE19. The expression of ADAM17 mRNA was increased in OE33, but did not differ in OE19.

ADAM protein expression in cell lines

The expression of the precursor and mature forms of ADAM proteins was analysed and shown for ADAMs 9 and 10 in Fig. 2.

For ADAM9, two precursor forms (at 110 kD and 100 kD), and two mature forms (at 80 kD and 75 kD) were observed. The expression the mature form at 80 kD was decreased in OE33 ($p = 0.0031$) and OE19 ($p = 0.0026$), and that of the mature form at 75 kD was increased in OE33 ($p < 0.001$) (Fig. 2a). For ADAM10, the expression of both the precursor form (at 80 kd) and the mature form (at 60 kD) were increased in OE33 (precursor form, $p = 0.0011$; mature form, $p < 0.001$) and OE19 (precursor form, $p = 0.0095$; mature form, $p < 0.001$), relative to the squamous control Het-1A (Fig. 2b). For ADAM12, only a precursor form was detected, and no change in its expression was seen (data not shown). For ADAM17, both a precursor form at 110 kD and a mature form ad 90 kD were detected, with no change in their expression, however (data not shown). For ADAM19, only small fragments of the protein were seen (data not shown).

ADAM mRNA expression in patient samples

The ADAM9 mRNA expression in distal esophagus differed between groups ($p = 0.002$), and was increased in BE ($p = 0.004$), BE with dysplasia ($p = 0.014$), and in esophageal adenocarcinoma ($p = 0.043$), relative to
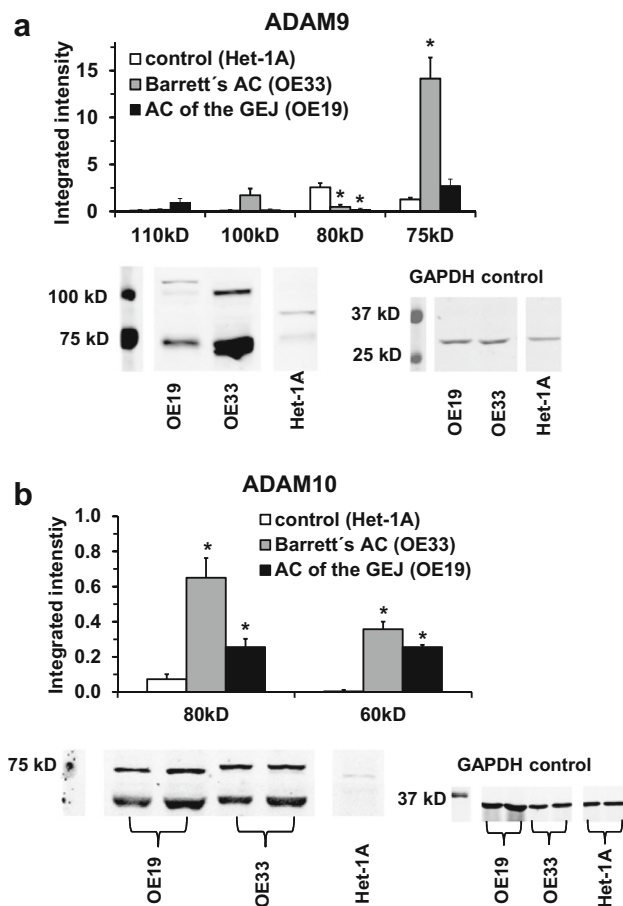
a

b

Fig. 2 Protein expression of ADAM9 **a** and ADAM10 **b** in esophageal cell lines by western blotting. A representative western blot, and the corresponding standardisation blot of GAPDH from the same nitrocellulose membrane, are shown below the densitometric results ($N = 4$–6). T test comparisons to Het-1A with significant difference ($p \leq 0.05$) are indicated with *stars*. *Error bars* represent SEM (standard error of mean). *AC* adenocarcinoma, *GEJ* gastro-esophageal junction

controls (Fig. 3a). The trend test showed a rising trend along the progressive stages of GERD from the control group to EAC ($p = 0.001$). When compared to proximal intrinsic controls, esophagitis ($p = 0.015$), BE ($p < 0.001$) and EAC ($p = 0.018$) showed increased ADAM9 expression.

The ADAM10 mRNA expression in distal esophagus varied between groups ($p = 0.007$), but the difference was not statistically significant for any single group (Fig. 3b). There was neither rising nor declining trend in the different stages of GERD. When compared to proximal intrinsic controls, ADAM10 mRNA expression in BE was higher than in these patients' proximal esophagus ($p = 0.001$).

The ADAM12 mRNA expression in distal esophagus differed between groups ($p < 0.001$). It was increased in esophagitis ($p = 0.038$), BE with dysplasia ($p = 0.001$),

and EAC ($p < 0.001$), relative to normal controls (Fig. 3c). In the trend test, there was a rising trend in ADAM12 expression ($p < 0.001$). In comparison to the proximal intrinsic controls, dysplastic BE ($p = 0.039$) and EAC ($p = 0.001$) showed increased ADAM12 expression.

The ADAM17 mRNA expression in distal esophagus, also, differed between groups ($p = 0.001$). It was increased in EAC ($p = 0.004$) (Fig. 3d). Neither rising nor declining trend during the GERD sequence was noted, and there were no differences relative to the proximal intrinsic controls.

The ADAM19 mRNA expression in distal esophagus differed between groups ($p = 0.002$). ADAM19 mRNA was increased in dysplastic BE ($p = 0.044$) and in EAC ($p = 0.001$) (Fig. 3e). The trend test showed a rising trend in ADAM19 expression ($p < 0.001$). When compared to proximal intrinsic controls, esophagitis ($p < 0.001$) and non-dysplastic BE ($p = 0.025$) showed increased ADAM19 expression.

Correlation with patient characteristics

The mRNA expression of ADAMs in the distal esophagus did not differ between the groups with or without neoad-juvant treatment (data not shown). Their expression was also not associated with cancer differentiation (good, moderate, poor) or dysplasia grade (mild, severe) (data no shown). ADAM9 expression was increased in Siewert1 cancers as compared to Siewert2 cancers (shown in Online Resource 2). ADAM12 was increasingly expressed in severe esophagitis as compared to mild esophagitis, as were also ADAM17 and ADAM19 (shown in Online Resource 2).

ADAM protein expression in patient samples

Normal esophageal squamous epithelium did not show distinct expression of ADAM 9, 10, or 17 proteins. ADAM12 was rarely seen in apically centred cytoplasmic granules. Only ADAM19 was mildly to moderately expressed in the cytoplasm of esophageal squamous cells. At whole, ADAM expression was centred in the glandular cells of the metaplastic/dysplastic esophageal epithelium, and was localized mostly in the cytoplasm but in EAC also occasionally in the nucleus.

ADAM9 was expressed in BE (Fig. 4c) and Barrett's dysplasia, with no increased staining relative to controls (for dysplasia, $p = 0.077$, median 1, range 1–1 in dysplasia vs. median 0.5, range 0–1 in controls) or from BE to dysplasia. EAC showed increased ADAM9 protein expression relative to controls (median 1, range 1–2 in EAC vs. median 0.5, range 0–1 in controls, $p = 0.026$) (Fig. 4a, d). ADAM9 was not differently expressed in esophagitis (Fig. 4b).
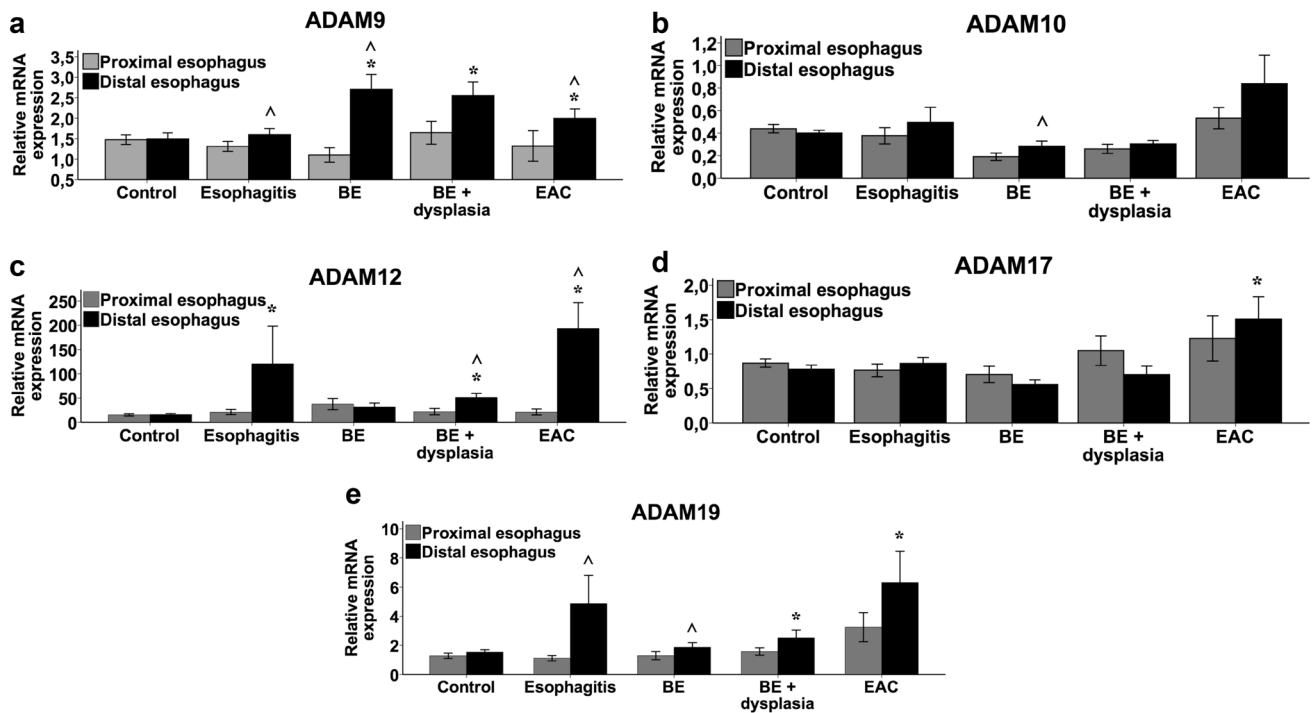
Fig. 3 ADAM mRNA expression in patient samples of different stages of GERD in both the distal and proximal esophagus. *Error bars* represent the standard error of mean. *Stars* indicate ANOVA/Dunnett's or Kruskal–Wallis/Dunn's test comparisons with significant difference ($p \leq 0.05$) between the patient group and controls, and *hat symbol* indicates paired t test or Wilcoxon signed rank test comparisons with significant difference ($p \leq 0.05$) between the distal and proximal samples of the same patient group. Values represent relative expression as compared to the expression in NCI-N87 cell line, corrected for the mean of GAPDH and beta actin expression in the same samples. ADAM9 (**a**) ADAM10 (**b**) ADAM12 (**c**) ADAM17 (**d**) ADAM19 (**e**)
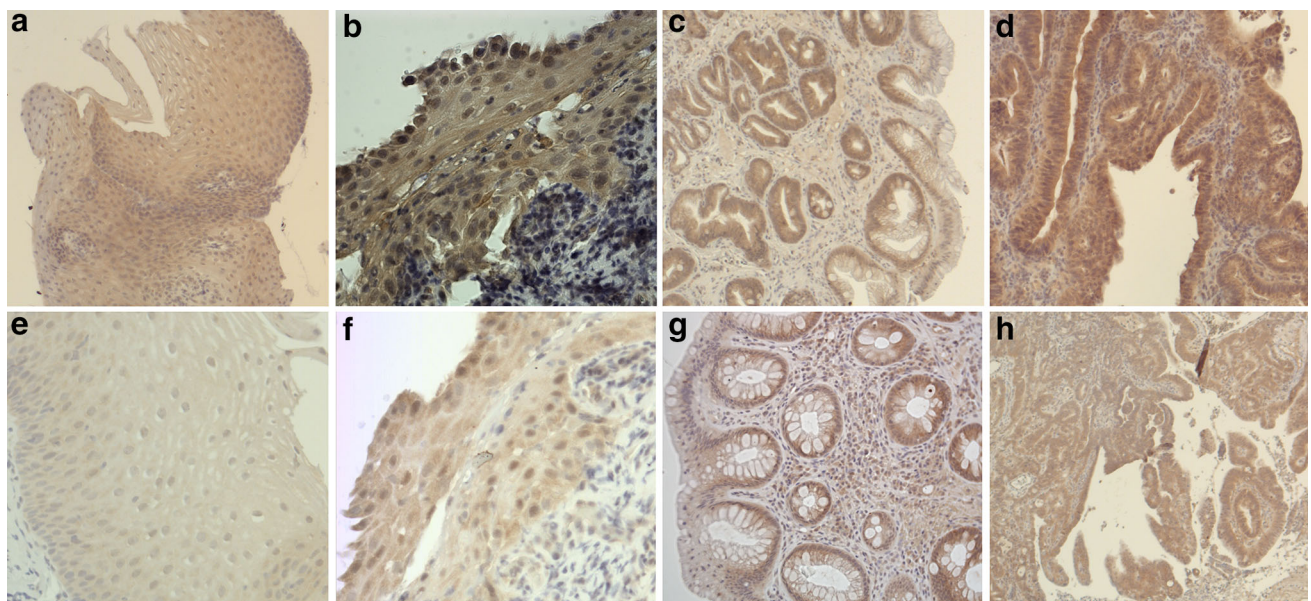

Fig. 4 ADAM9 protein expression in normal squamous mucosa of distal esophagus (original magnification ×100) (**a**), in esophagitis (×200) (**b**), in Barrett's esophagus (×100) (**c**), and in esophageal adenocarcinoma (×100) (**d**). ADAM10 expression in normal squamous mucosa of distal esophagus (×200) (**e**), in esophagitis (×200) (**f**), in Barrett's esophagus (×100) (**g**) and in esophageal adenocarcinoma (×40) (**h**)

ADAM10 expression showed a tendency for basal localisation in BE, while in Barrett's dysplasia its expression was more evenly distributed. In EAC, ADAM10 was increasingly expressed relative to controls (Fig. 4e, h) (median 2, range 1–2 in EAC vs. median 1, range 0–1 in controls, $p = 0.021$), while for dysplasia there was no increase (median 1, range 1–2 in dysplasia vs. median 1, range 0–1 in controls, $p = 0.061$), and neither for BE (Fig. 4g). ADAM10 was not differently expressed in esophagitis (Fig. 4f).

The significant increase of ADAM12 mRNA in severe esophagitis was not reflected at the protein level. Statistically, ADAM12 expression did not differ from normal esophageal squamous epithelium at any stage of GERD.

ADAM17 was increasingly expressed in BE relative to normal controls (median 2, range 2–2 in BE vs. median 0.5, range 0–1 in controls, $p = 0.024$), while for Barrett's dysplasia or EAC there was no difference in the expression relative to controls.

ADAM19 was expressed at all stages of GERD (including severe esophagitis), with a tendency for transformation from basally centred staining in BE to apically centred staining in EAC. Statistically, however, the ADAM19 expression in BE/dysplasia/EAC did not differ from the controls.

Discussion

To our knowledge, this is the first study to examine the ADAM expression in the various phases of GERD preceding cancer, and in EAC also at the protein level. ADAM9 was increased in EAC, and in BE and BE with dysplasia at mRNA level. Other ADAMs studied showed alterations as well, and may participate in the complex network of interactions leading to EAC development.

In EAC, both ADAM9 mRNA and protein expression were elevated. Also in the cancer cell lines, ADAM9 mRNA was elevated, and in the Barrett's adenocarcinoma cell line, ADAM9 protein was elevated. The demonstrated functions of ADAM9 in cancer include promoting cellular adhesion and invasion, as well as interaction with integrins [7]. It can bind to the α6β4-integrin, the α6-subunit of which is upregulated in EAC [7, 17]. ADAM9 has been found increased e.g. in gastric and prostate cancer, and in pancreatic ductal adenocarcinoma [18–20]. There is preliminary evidence of ADAM9 being involved in invasion/metastasis [21–23]. Oxidative stress, which is also increased in EAC and its predecessors [24], induces the expression of ADAM9 [20], which also contributes to the cellular invasion induced by oxidative stress [25]. Given the potential contribution of ADAM9 for the cancer metastasising, and the availability of an inhibitor [7], it

remains an interesting candidate for further studies assessing its function in GERD and EAC.

ADAM10 protein but not mRNA expression was increased in EAC both in the cell lines and in human samples. This might be caused by some posttranscriptional or posttranslational modulation leading to e.g. increased translation of mRNA, or decreased degradation of the protein. As shown before for ADAM17, posttranscriptional mechanisms may alter ADAM protein expression without a change in mRNA level by altering the protein half-life [26]. Previously, however, an increase in ADAM10 mRNA has been found in esophago-gastric carcinomas [14]. ADAM10 expression has been linked to many human cancers [7]. Possible mechanisms for promoting carcinogenesis in EAC include its ability to cleave tumour necrosis factor alpha (TNF-α) [27], increased along the metaplasia—dysplasia—carcinoma—sequence [28], and E-cadherin [29], leading to increased free ß-catenin levels, and thereby increased expression of the proto-oncogene C-myc, also found upregulated in Barrett's adenocarcinoma [28, 30]. It might also act by cleaving the adhesion molecule CD44 [7], also shown to be associated with EAC [17].

In human samples, ADAM12 mRNA was increased in esophagitis, BE with dysplasia, and in EAC. ADAM19 showed a similar expression profile, except for that in esophagitis and BE it was increased only relative to intrinsic controls. These elevations were not, however, seen at the protein level. It is possible that in these tissues the prevailing form of these ADAMs was not captured by our IHC protocol. ADAM12 may exist as a membrane-anchored long form, or as a soluble secreted form [31]. As ADAM12 and ADAM19 mRNA were highly expressed in esophagitis, their increased expression in EAC might reflect the inflammatory changes seen, and possibly of importance for the development of a malignancy, such as EAC [32, 33]. Our results are in accordance with the previous study of esophago-gastric adenocarcinomas [14]. ADAM12 and ADAM19 expression have been linked to both inflammatory diseases and cancer [7, 11, 31, 34, 35].

ADAM17 mRNA, but not protein, was increased in EAC in human samples, and in the Barrett's adenocarcinoma cell line OE-33. IHC may not have captured the relatively small increase in mRNA. Tumours also showed marked heterogeneity in the expression of ADAM17 protein. Previously, ADAM17 mRNA has been found increased in esophago-gastric adenocarcinomas [14]. This molecule is one of the most studied ADAM-molecules and is upregulated in many human cancers [7]. It cleaves TNF-α, and also TGF-β, both cytokines of possible importance in EAC [7, 13, 36].

We compared the ADAM expression in GERD and EAC to both normal controls and to the proximal unaffected

mucosa of the patients. The previous study of esophago-gastric adenocarcinomas compared the ADAM expression in cancer to "peri-tumoural normal tissue" [14]. We used statistical correction for the multiple comparisons problem, which may have affected our results in comparison to studies without these corrective methods. Our results were, however, mostly in accordance with the previous study showing increased ADAM mRNA expression in esophago-gastric adenocarcinomas [14].

A limitation of our study was the relatively small number of samples, especially in the preliminary characterisation of ADAM expression at the tissue level by IHC. The elevated ADAM mRNA levels were not always seen at the protein level. This may be at least partly due to ADAMs being subjects to significant post-transcriptional and post-translational regulation [8]. This kind of modulation, leading possibly to altered levels of specific isoforms, altered function, or altered localisation, may even be an important regulatory mechanism controlling the cellular and inter-cellular actions of ADAMs.

The major disparities between ADAM expression in the cell lines studied and the human samples are not surprising, given the individual nature of molecule expression in each cancer and heterogeneity across each tumour [37]. In human samples, a factor likely to affect the molecular expression is the stroma and its interactions with the cancer cells [38]. With the small number of samples, neoadjuvant treatment did not affect ADAM expression. Our patients were a little older than the controls, and there was a male predominance in patients in contrast to the male proportion of only 35 % in the controls. This is likely to mostly reflect the age and gender as known risk factors of BE and EAC [39]. To our knowledge, no reports of age- or gender-dependent differences in ADAM expression in the adult have been reported, but a possible bias cannot be excluded.

We have shown that the expression of this multifunctional ADAM protein group is altered in EAC and during its development. ADAMs are linked to inflammatory processes and to many molecules already shown to be dysregulated along the development of EAC. Even a minor modification in the expression of regulatory molecules, such as ADAMs, may have a significant impact. Future studies with more samples, data on protein levels, association with prognostic factors and fate, and functional assays, are needed to define the role of ADAMs in esophageal carcinogenesis.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Informed consent Informed consent was obtained from all individual participants included in the study.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

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