FEBS Letters 587 (2013) 3529–3534

FEBS Letters

journal homepage: www.FEBSLetters.org

ADAM 10 is over expressed in oral squamous cell carcinoma and contributes to invasive behaviour through a functional association with αvβ6 integrin

Adam V. Jones [1], Daniel W. Lambert, Paul M. Speight, Simon A. Whawell *

Academic Unit of Oral & Maxillofacial Pathology, School of Clinical Dentistry, University of Sheffield, UK

ARTICLE INFO

Article history:
Received 8 August 2013
Revised 5 September 2013
Accepted 10 September 2013
Available online 18 September 2013

Edited by Gianni Cesareni

Keywords:
ADAM 10
αvβ6 integrin
Oral squamous cell carcinoma

ABSTRACT

A disintegrin and metalloprotease (ADAM) proteins are upregulated in cancer and can interact with integrin receptors. We investigated whether such interactions may have functional significance in oral squamous cell carcinoma (OSCC).
ADAM 10 expression was increased in OSCC tissue and cell lines compared to normal oral mucosa. Silencing of ADAM 10 reduced migration and invasion specifically in OSCC cells over-expressing αvβ6 integrin. This may result from ADAM 10-induced up-regulation of MMPs.
We conclude ADAM 10 may influence OSCC invasion by functionally interacting with αvβ6 integrin which we have previously shown is over expressed in OSCC.

1. Introduction

A disintegrin and metalloprotease (ADAM) are a family of transmembrane proteins which orchestrate interactions with the ECM by proteolytically modifying cell surface proteins and acting as adhesion molecules [1]. ADAM 10 is essential for normal development and for differentiation of epithelial cells [2] largely through Notch signalling [3]. ADAM 10 sheds numerous cell surface proteins including TNFα [3] HB-EGF [4], TGFα [5] and EGF [6] and is capable of mediating regulated intramembrane proteolysis (RIP) of CD44 [7], cadherins [8], and notch [9], which leads to the production of an intracellular fragment that can translocate to the nucleus and influence gene transcription [10].

Despite advances in therapy the general prognosis of oral squamous cell carcinoma (OSCC) remains poor, highlighting the need for increased understanding of the molecular mechanisms involved in disease progression. Over-expression of ADAM family members has been reported in a range of malignancies [11–16] including those of the oral cavity. ADAM 10 shows has proliferative effects on OSCC cell lines [17] but the mechanism by which this occurs is unclear.

The pattern of expression of a number of integrins is changed in cancer supporting the hypothesis that such molecules are intimately involved in tumour progression [18]. The importance of the integrin αvβ6 in OSCC has been widely reported [19,20]. Over-expression of this integrin and engagement to its ligand fibronectin on the surface of keratinocytes is thought to initiate a pro-migratory cascade including up-regulation of MMP-2 and 9 [21,22].

Given that expression of ADAM 10 and integrin αvβ6 are altered in OSCC and that interactions between integrins and ADAMs have been shown to have functional significance, we aimed to investigate this further. We demonstrate that expression of ADAM 10 is increased in OSCC and that reducing its expression by siRNA modified cell motility specifically in cells over expressing the αvβ6 integrin. Our data suggests one potential consequence of this functional association is the modification of MMP expression as a result of engagement of αvβ6 with ADAM 10.

2. Materials and methods

2.1. Patient samples

Tissue samples comprised 61 normal oral mucosa, 29 epithelial dysplasias and 50 oral squamous cell carcinomas (25 cases with and 25 without metastasis). Tissue micro-arrays were prepared following examination of haematoxylin and eosin stained slides and areas of interest marked using an objective marker (1.8 mm,

* Corresponding author. Address: Academic Unit of Oral & Maxillofacial Pathology, School of Clinical Dentistry, University of Sheffield, Claremont Crescent, Sheffield S10 2TA, UK.
E-mail address: s.whawell@sheffield.ac.uk (S.A. Whawell).
[1] Current address: School of Dentistry, University of Cardiff, Heath Park, Cardiff, UK.

0014-5793/$36.00
http://dx.doi.org/10.1016/j.febslet.2013.09.010

Nikon Corporation). Three tissue cores measuring 1 mm in diameter and 3 mm in depth were harvested from each sampled area using a manual tissue array (MTA-1 Mitogen, Beecher instruments Inc.) and placed in a recipient paraffin block. For the OSCC cases, cores were obtained from histologically normal mucosa, and from the origin, body and advancing front of the tumour.

2.2. Immunohistochemistry

Sections were deparaffinised using xylene and ethanol and antigen retrieval performed by microwaving for 8 min in Tris–EDTA buffer.

Slides were washed in PBS and endogenous peroxide blocked using 3% (v/v) H_2O_2 in methanol for 20 min. Non-specific blocking was performed for 30 min with 100% (v/v) normal goat serum. ADAM 10 antibody (Abcam; diluted 1:100 in goat serum) was applied to the sections and incubated overnight at 4 °C. Secondary anti-rabbit horseradish peroxidase-conjugated antibody, immunoperoxidase kits and Vector NovaRed (VectorLabs), were used according to manufacturer's instructions. Slides were counter stained with haematoxylin and mounted. Quantitative analysis was performed using an Automated Cell Imaging System (ACIS III, Dako). Initially, conventional microscopic analysis of approximately 10% of the cases was performed visually and scored according to a modified Allred scoring system [23]. Threshold levels were optimised within the ACIS III scoring system using the 'learn by example' tool.

2.3. Cell culture and siRNA treatment

Three oral cancer cell lines [22] were selected with defined integrin expression patterns: H357 (negative for the αv and β6 integrin subunits), C1 (αv positive, β6 negative) and VB6 (αvβ6 positive) and cultured in keratinocyte growth medium as previously described [24]. Integrin expression patterns for αvβ6 were confirmed by flow cytometry [25] using monoclonal antibody MAB2077Z (Millipore). Transfection of synthetic siRNA were performed as previously described [30], using oligofectamine (Life Technologies) with ADAM 10 siRNA or a control siRNA (Life Technologies, final concentration 50 nM). Normal oral keratinocytes (NOK) were prepared from outgrowth cultures of mucosal biopsies with appropriate ethical approval.

2.4. mRNA and protein analysis

RNA was extracted using Trizol (Sigma–Aldrich) according to the manufacturer's instructions. Samples were treated with DNAase I and cDNA generated using MultiScribe (Stratagene) reverse transcriptase and qPCR performed using ADAM 10 and β2-microglobulin specific TaqMan gene expression assays (Applied Biosystems). Protein extraction was performed with RIPA buffer (Sigma Aldrich) containing protease inhibitors (Roche) and western blot analysis performed as described previously [26]. Primary antibodies for ADAM 10 (Calbiochem) and β-actin (Sigma–Aldrich) were used at dilutions of 1:2000 and 1:5000 respectively. Blots were visualised using GeneSnap (Syngene) and densitometric analysis performed by comparison with β-actin expression using Image J (National Institute of Health, USA).

2.5. Adhesion assays

Ninety six well tissue culture plates (Corning) were coated overnight at 4 °C with either 10 μg/ml purified plasma fibronectin (Sigma–Aldrich) or PBS, then blocked with 1% (w/v) BSA for 1 h. 4×10^4 cells were resuspended in serum free media and plated in triplicate for 1 h at 37 °C. Wells were washed, 100 μl of SFM with 20 μl of MTS (Fisher Scientific, UK) added and absorbance read at 492 nm.

2.6. Migration assays

Migration chambers (8 μm pores, Corning) were coated for 1 h at 37 °C with either 10 μg/ml purified plasma fibronectin or PBS and blocked with migration buffer (3:1 of DMEM:F12 and 0.5% (w/v) BSA) for 1 h. 1×10^5 cells were resuspended in 100 μl of migration buffer, placed in the chambers and incubated at 37 °C for 4 h. Non-migrated cells were removed, samples fixed in 10% (v/v) buffered formalin, stained with haematoxylin and mounted on glass slides. The number of migrated cells was determined by counting five randomly selected fields.

2.7. Invasion assays

Matrigel coated invasion assays (BD Biosciences) were performed using triplicate samples seeded at 3×10^5 cells/ml in low serum media (3:1 of DMEM:F12 with 0.5% (v/v) FCS and 2% (w/v) L-glutamine) in the inner chamber and KGM was used as a chemo-attractant. Plates were incubated for 16 h at 37 °C and the invaded cells processed and counted as above.

2.8. MMP assays

24 well culture plates were coated with 10 μg/ml of recombinant ADAM 10 (R&D Systems), purified fibronectin or PBS and blocked with 0.1% (w/v) BSA at 37 °C for 30 min. Serum-starved cells were cultured until 90% confluent. Media was removed, cells washed and replaced with SFM for 24 h. Subsequently, the media was removed, centrifuged and protein concentration determined using a BCA assay (Thermo Scientific) and the number of adherent cells counted using an automated cell counter (The Countess, Invitrogen). Electrophoresis using 10% (w/v) SDS–PAGE gels supplemented with 1 mg/ml of porcine gelatine was performed using cell supernatants containing equal protein and MMP-2 and 9 standards (Millipore) diluted 1:400. Gels were re-natured in buffer containing 2.5% (v/v) Triton X-100 and incubated overnight at 37 °C in developing buffer as previously described [27]. Coomassie Blue R-250 stained gels were scanned and analysed for MMP-2 and 9 activity by densitometry using Image J. MMP-2, 7 and 9 were quantified by ELISA according to manufacturers' instructions (R&D Systems).

2.9. Statistical analysis

Kruskall Wallis and one way analysis of variance tests were performed on cell-based and immunohistochemical data respectively, using Analyse-It (Analyse-It Software Ltd., UK).

2.10. Ethics

Ethical approval for collection of primary normal oral keratinocytes (REC reference number: 04/Q2305/78) and for ADAM protein immunohistochemistry on tissue sections (REC reference number: 07/H1309/105) was given by South Sheffield Research Ethics Committee.

3. Results

3.1. ADAM 10 is significantly increased in OSCC compared to normal oral mucosa and epithelial dysplasia

Most of the tissue cores examined demonstrated intense staining for ADAM 10, with the majority showing cytoplasmic staining