SUPPLEMENTARY METHODS

Clinical characterisation

Asthmatic subjects and non-asthmatic controls were recruited from Leicester, UK. Asthmatic subjects had a consistent history and objective evidence of asthma.[1] Asthma severity was defined by Global Initiative for Asthma treatment steps (mild-moderate GINA 1-3, severe GINA 4-5).[1] Subjects underwent extensive clinical characterisation including allergen skin prick tests, spirometry, methacholine bronchial challenge (PC20), sputum induction, and video-assisted fibreoptic bronchoscopic examination (Table E5). The study was approved by the Leicestershire Ethics Committees and all patients gave their written informed consent.

Culturing conditions for the differentiated NHBEC model

Commercial cells were cultured on polyester inserts with a pore size of 0.4μm at air-liquid interface (ALI) according to a previously published method.[2, 3] Briefly, cells were plated at 100,000 cells per insert in BEDM. When confluent (~5 days), cells were raised to ALI. Medium was replaced and the apical face washed with PBS every 48 hours. Experiments were performed after 21 days at ALI. BEDM is composed of 50:50 DMEM:BEBM with Lonza singlequots, excluding triiodo-L-thyronine and retinoic acid. Medium is supplemented with 50 nM retinoic acid, added fresh at time of use. Cells at ALI were characterised by immunostaining for goblet cell (MUC5AC) and ciliated cell (beta-tubulin IV) markers, as well as staining for F-actin (Figure E2).

Wound repair experiments in NHBECs

For wounding experiments in monolayer cultures, confluent cells in a 6-well plate were wounded using a p200 pipette tip with either 3 wounds for imaging purposes or 5+5 wounds in a cross-hatch pattern for analysis of signaling events. Twenty-four hours prior to wounding, culture medium was replaced with growth medium excluding epidermal growth factor (EGF), hydrocortisone and epinephrine, as these or related molecules have previously been shown to

modulate wound repair [3, 4] and uPAR expression.[5] Wound repair was visualised over 24 hours at nine sites in each well using the Nikon Diaphot 300 Inverted Microscope (4x objective). Wound areas were calculated using Spot 4.6 software (Diagnostic Instruments, MI, USA). Data are shown as area of wound healed and for comparison between experiments are normalised to area healed at 8 hours in untreated or control cells. This measurement was shown to reflect changes in rate of migration without being affected by experimental variation in the initial wound area. For wounding experiments at ALI, two wounds were made per insert and repair visualised at one site per wound, these experiments used three replicate wells per condition (i.e. six images). To block the uPA-uPAR interaction, anti-uPA (394OA, American Diagnostica, Axis-Shield UK, Cambridge) was added to the medium at time of wounding (10-40µg/ml). An isotype control IgG antibody (sc-2025, Santa Cruz Biotechnology, Heidelberg, Germany) and an isotype-matched anti-uPAR domain II antibody (3932, American Diagnostica) which was anticipated not to affect the uPA-uPAR interaction were used as controls.

Preparation and transfection of plasmids

The open reading frame of membrane uPAR (NM_002659.3) was amplified using primers including a consensus Kozak sequence and restriction enzyme sites (5'primer: ACTTGAATTCGCCACCatgggtcacccgccgct spacer-EcoRI-Kozak-ATG. 3'primer: ACTTCTCGAGttaggtccagaggagagtgc spacer-XhoI-stop). PCR product was cloned into pCR4-TOPO (Invitrogen) then subcloned into pcDNA3 (Promega, Southampton, Hampshire) using appropriate restriction enzymes and T4 ligase (Promega). Plasmid was sequence verified before transfecting into NHBECs at 80% confluence using a 3:2 ratio of Fugene 6 (Roche) to plasmid according to manufacturer's instructions. Medium was replaced after 24 hours, when further experiments were performed.

Gelatin zymography

Activity of MMP2 and 9 in cell culture supernatants was analysed by gelatin zymography as described previously.[6] Aliquots of medium (10µl) diluted 1:1 in SDS loading buffer were subject to electrophoresis on pre-cast gelatin gels (Invitrogen) in triplicate. Gels were soaked in renaturing buffer for one hour and developing buffer at 37°C overnight, before staining with Coomassie blue. Activated recombinant MMP2 and supernatant from a cell-line engineered to overexpress MMP9 were used as both molecular weight markers and loading controls. Gels were imaged using the GeneGenius Gel Doc and GeneSnap 7.07 software (SynGene, Cambridge, UK). Densitometry of .tif images used ImageJ 1.41 (http://rsbweb.nih.gov/ij/) and data were normalised to the recombinant MMP2 control. Using a dilution series of recombinant MMP2, we showed a semi-quantitative relationship between MMP concentration and densitometry (Figure E3).

References

1. O'Byrne PM. Global guidelines for asthma management: summary of the current status and future challenges. Pol Arch Med Wewn. 2010 Dec;120(12):511-7.

2. Danahay H, Atherton H, Jones G, Bridges RJ, Poll CT. Interleukin-13 induces a hypersecretory ion transport phenotype in human bronchial epithelial cells. Am J Physiol Lung Cell Mol Physiol. 2002 Feb;282(2):L226-36.

3. Wadsworth SJ, Nijmeh HS, Hall IP. Glucocorticoids increase repair potential in a novel in vitro human airway epithelial wounding model. J Clin Immunol. 2006 Jul;26(4):376-87.

4. Sivamani RK, Pullar CE, Manabat-Hidalgo CG, Rocke DM, Carlsen RC, Greenhalgh DG, et al. Stress-mediated increases in systemic and local epinephrine impair skin wound healing: potential new indication for beta blockers. PLoS Med. 2009 Jan 13;6(1):e12.

5. Baek MK, Kim MH, Jang HJ, Park JS, Chung IJ, Shin BA, et al. EGF stimulates uPAR expression and cell invasiveness through ERK, AP-1, and NF-kappaB signaling in human gastric carcinoma cells. Oncol Rep. 2008 Dec;20(6):1569-75.

6. Roberts ME, Magowan L, Hall IP, Johnson SR. Discoidin domain receptor 1 regulates bronchial epithelial repair and matrix metalloproteinase production. Eur Respir J. 2010 Sep 30.

Table E1: HBEC donor information, taken from Lonza datasheets.

Donor	Age	Gender	Race	Smoking status	Doubling time (hours)
4F0507	51	Female	Asian	Not known	15
7F3158	56	Male	Caucasian	Yes	24.5
7F3206	50	Male	Caucasian	Yes	21

Table E2: Immunofluorescent staining of cultured cells

target	antibody	dilution	secondary
uPAR	Santa Cruz Biotechnology IIIF10/sc-32764	1:50	goat anti-mouse (Invitrogen A11001) 1:250
MUC5AC	Thermo Scientific Ms-10331-P	1:1500	
beta-tubulin IV	Sigma T7941	1:1500	
F-actin	Alexa488-phalloidin (Fisher VXA12379)	1:40	NA

Table E3: Details of primers and TaqMan probes for QPCR

Target	Primer1	Primer2	Probe
uPAR	CTGCTGCTGCTCCACACCT	ACTCTTCCACACGGCAATCCC	CCAGCCTCTTGGGGCCTGCGGT
uPA	AACCTCATCCTACACAAGGACTAC	GGCAGGCAGATGGTCTGTATAG	CGCTGACACGCTTGCTCACCACAA
PAI1	AAGACTCCCTTCCCCGACTC	GGGCGTGGTGAACTCAGTATAG	ACCCACCGCCGCCTCTTCCACA

Table E4: Antibodies used for Western blotting

Target	Antibody	Dilution	Secondary
uPAR (45-55kDa)	IIIF10/sc-32764 Santa Cruz Biotechnology	0.25µg/ml 1:400 1% goat serum	HRP conjugated anti-mouse (R&D systems)
AKT (60kDa)	9272, CST	1:1000 5% BSA	anti-rabbit
p^{Thr308}-AKT	9275, Cell Signaling Technology (CST)	1:1000 5% BSA	HRP conjugated anti-rabbit (R&D systems)
ERK1/2 (42/44 kDa)	9102, CST	1:1000 5% BSA	anti-rabbit
$p^{Thr202/Tyr204}$-ERK1/2	9106, CST	1:1000 5% BSA	anti-mouse
p38MAPK (43 kDa)	9212, CST	1:1000 5% BSA	anti-rabbit
$p^{Thr180/Tyr182}$-p38MAPK	9211, CST	1:1000 5% BSA	anti-rabbit
Beta-actin (47kDa)	ab8227, Abcam	1:5000 0.2% BSA	anti-rabbit

Table E5: Details of asthma patients and controls. Mean and SD shown for age, smoking pack-years and FEV_1 (% pred), median and range for PC20 and BDP Eq.

	Controls	Mild-moderate asthma (GINA 1-3)	Severe asthma (GINA 4-5)
Number	9	15	12
Female (n)	5	7	6
Age (SD)*	36.4 (13.1)	48.0 (17.4)	50.1 (13.1)
Smoking PY (SD)	0.11 (0.33)	2.70 (3.82)	2.50 (3.61)
Atopy (n)	4	9	9
FEV_1 (% pred) (SD)	97.3 (11.1)	89.6 (18.9)	80.2 (22.8)
PC20 (range)	>16 (>16->16)	0.44 (0.013-3.5)	0.76 (0.03-1.5)
BDP Eq	0 (0-0)	0 (0-2000)	1120 (640-2240)
Po CS (n)	0	0	3
LABD (n)	0	2	12

Abbreviations: SD: standard deviation, PY: pack years, FEV_1 (% pred): FEV1 percent predicted, PC20: Provocation concentration of inhaled methacholine required to reduce FEV1 by 20%. BDP Eq: Beclometasone Dipropionate equivalent dose of inhaled corticosteroids. Po CS (n): number of individuals prescribed oral corticosteroids. LABD (n): number of individuals prescribed long-acting bronchodilators.

ONLINE SUPPLEMENT FIGURE LEGENDS

Figure E1: NHBECs repair a scratch wound within 24 hours. Representative images of wound repair in undifferentiated (A) and ALI differentiated (B) NHBECs over 24 hours.

Figure E2: Characterisation of ALI differentiated NHBECs. Cells differentiated at ALI show expression of beta-tubulin IV (Ai), at the apical side of the pseudostratified layer (side projection, Aii), and MUC5AC (B) in a subset of cells (arrowed). Cells show localised actin expression (C), this image was taken 4 hours post-wounding.

Figure E3: Semi-quantiative zymography. A dilution series of activated recombinant human MMP2 was performed (A) and densitometry measured (B). This showed a semi-quantitative relationship between densitometry and MMP2 concentration. Supernatants were taken from differentiated cells at 0, 4, 8 and 24 hours after wounding (W) or from unwounded controls (U). Zymography was performed using activated recombinant MMP2 (MMP2) and supernatant from an MMP9 over-expressing cell line (MMP9) as controls (C). Pro-MMP2 and 9 were detected and measured using densitometry.