

Increased alveolar soluble Annexin V promotes lung inflammation and fibrosis

Supplemental data - Materials and methods

Human BALF. Bronchoalveolar Lavage (BALF) was performed at the National Institute of Respiratory Diseases (INER), Mexico City, Mexico, as approved by the Ethics Committee at INER. It is performed through flexible fiberoptic bronchoscopy under local anesthesia. Briefly, 200 ml of normal saline is instilled in 50-ml aliquots, with an average recovery of 60%–70%. The cells are pelleted from the BALF, and the cell-free supernatant is frozen at -80°C . The control group for Figure 1 included 3 healthy volunteers, and 2 individuals without HRCT-scan evidence of lung disease who required a bronchoscopy for the diagnosis of unexplained cough. In all cases bronchoscopy did not display any abnormality.

Removal of Annexin V from BALF by immunoprecipitation. BALF samples with known elevated Annexin V levels were pre-cleared by mixing with $1\mu\text{g}$ rabbit IgG and $20\mu\text{l}$ of protein A/G agarose at 4° for 30 mins, followed by centrifuging at $1000g$ for 30s at 4°C . The supernatants were removed to clean tubes and incubated with mixing for 1h with $4\mu\text{g}$ rabbit anti-Annexin V antibody or $4\mu\text{g}$ rabbit IgG, then $20\mu\text{l}$ of protein A/G agarose was added and the tubes were incubated with mixing for 1h at 4°C . The tubes were centrifuged for 30s at $1000g$, and the supernatant carefully removed. PBS washes of the immunoprecipitate were pooled with the supernatant. Successful reduction of Annexin V levels was confirmed by ELISA.

Intra-tracheal instillation of Annexin V solution. 8 week C57 female black mice were anaesthetized with isoflurane and suspended at a $45\text{-}60^{\circ}\text{C}$ angle on an intubation platform. The oropharynx and larynx were visualized with a small animal laryngoscope (LS-2; Penn-Century), and $150\mu\text{l}$ of Annexin V or vehicle (20mM phosphate buffer, 0.02% Tween 80, 130 mM arginine HCl), both diluted in PBS, was then aerosolized and delivered into the airway using a microsyringe tip which was inserted past the vocal cords and into the trachea. The tip was left inside the trachea for a few seconds, then the mouse was taken off the support platform and laid supine on a heating source until the effects of anaesthesia wore off. To mimic a limited chronic exposure, Annexin V was administered to mice twice a week for 2 weeks. The $150\mu\text{l}$ aerosol administered to each mouse contained < 0.006 EU, 4% of the maximum acceptable endotoxin that can be administered to a 30g mouse (20).

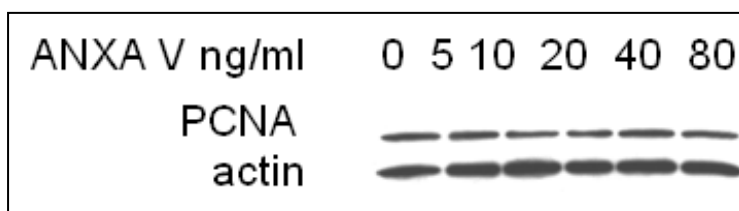
Quantitation of soluble collagen in conditioned medium. Collagen was precipitated from cell-conditioned medium, medium alone, or collagen standards assayed in parallel, by 30 min treatment with 0.1% Sirius Red in saturated picric acid (1ml dye to $50\mu\text{l}$ CM). The collagen was pelleted by centrifuging the samples at $10,000g$, and the supernatants were carefully discarded. The pellets were

washed with 0.1M HCl to remove unbound dye, and re-centrifuged. The dye was then eluted from the pellets by vigorously vortexing with 0.5M NaOH, then the solutions were transferred to a 96 well plate and the absorbance measured at 540nm in a plate reader. Values were calculated from a standard curve from collagen standards assayed in parallel.

Supplementary data - Results

Figure 1. Annexin V does not induce fibroblasts to proliferate

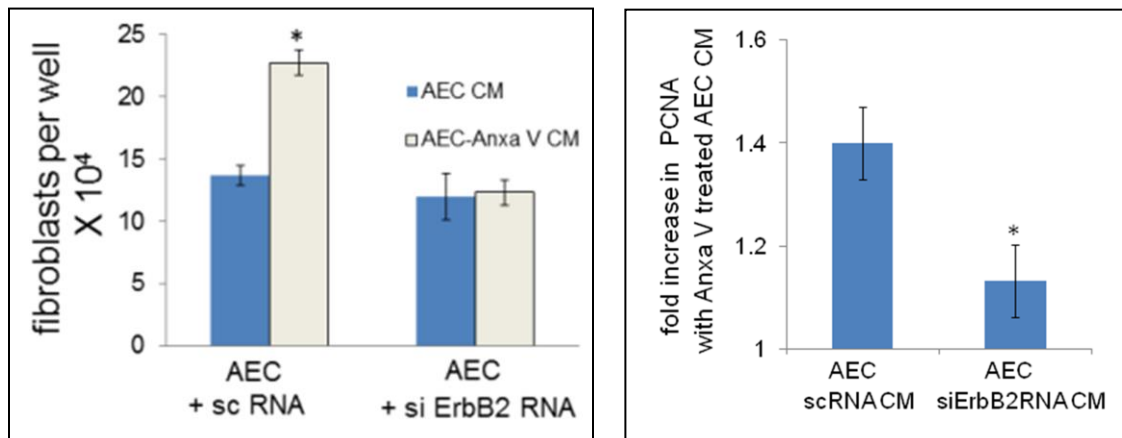
Human fibroblasts (WI38 from ATTC) were plated at a density of 5×10^4 cells per cm^2 , and cultured for $24\text{h} \pm 50$ ng/ml Annexin V. The cells were then trypsinized, neutralized and counted. Three independent experiments showed no difference in cell counts: untreated: $9.3 \pm 2.2 \times 10^4$ cells/well versus Annexin-treated $9.2 \pm 2.4 \times 10^4$ cells/well. These experiments were repeated with embryonic mouse fibroblasts (NIH3T3 from ATTC) and an adult human fibroblast cell line (CCD-8Lu from ATTC), with the same results (not shown).



Lysates of WI38 lung fibroblasts that had been treated for 24h with increasing doses of Annexin V, added at the time of plating, were analysed by western blot for PCNA expression,

corrected for actin. PCNA was not induced with Annexin V treatment. Results were similar when embryonic mouse fibroblasts (NIH3T3) or human fibroblast cell line CCD-8Lu were treated as above (not shown).

Figure 2. CM from Annexin V-treated AEC (MLE15) is less stimulatory to human adult fibroblasts when ErbB2 is silenced in the AEC, as measured by fibroblast cell counts and PCNA.

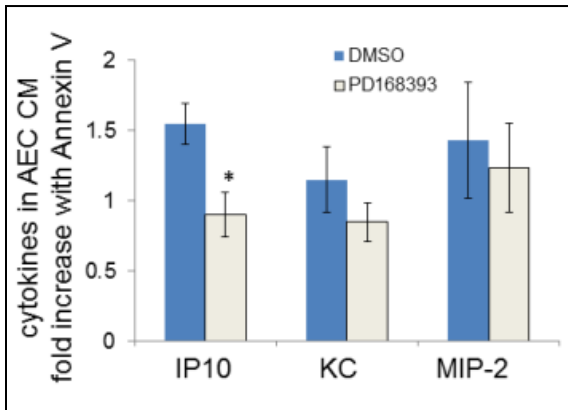


Left panel shows that. Annexin V treatment of AEC in the presence of 10 nM scrambled RNA resulted in CM that induced a 1.7 fold stimulation in fibroblast growth, as measured by fibroblast cell counts

after 24h treatment with the CM ($P < 0.01$ by ANOVA, followed by Tukey's post hoc test). The Annexin-mediated growth response was abolished when AEC were treated with Annexin V in the presence of 10 nM silencing RNA to ErbB2.

Right panel shows decreased PCNA expression in adult human fibroblasts when treated with CM from ErbB2-silenced, Annexin V treated AEC, versus CM from Annexin V-treated, scrambled RNA treated AEC, as measured by band densitometry, corrected for actin, from 3 western blots, $P < 0.02$.

Figure 3. . ErbB RTK inhibition blocks Annexin V-mediated IP10 release from MLE15 AEC, but not the release of KC and MIP-2



MLE15 AEC were treated for 2h \pm 10 μ M ErbB tyrosine kinase inhibitor PD168393, then Annexin V, 50 ng/ml, was added for 24h. Cytokine analysis of CM from 3 independent treatments using mouse cytokine protein arrays (R&D, Minneapolis, MN), showed that Annexin V-mediated IP10 secretion was reduced by PD168393, $p < 0.03$, $n = 3$, while secretion of the other 2 cytokines induced in AEC2 by Annexin V, KC and MIP2, was not altered.

Table 1. Proteomic screen of epithelial-healing versus pro-fibrotic healing human BALF

Protein Name	Accession Number	MW	Human BALF		
			Protein Grouping	pro-	pro-
				epithelial healing	fibrotic healing
1 Serum albumin OS=Homo sapiens GN=ALB PE=1 SV=2 [MASS=69366]	ALBU_HUMAN	69 kDa		968	778
2 Serotransferrin OS=Homo sapiens GN=TF PE=1 SV=2 [MASS=77049]	TRFE_HUMAN	77 kDa		71	75
3 Ig gamma-1 chain C region OS=Homo sapiens GN=IGHG1 PE=1 SV=1 [MASS=36106]	IGHG1_HUMAN	36 kDa	TRUE	33	52
4 Ig kappa chain C region OS=Homo sapiens GN=IGKC PE=1 SV=1 [MASS=11609]	IGKC_HUMAN	12 kDa		30	63
5 Ig alpha-1 chain C region OS=Homo sapiens GN=IGHA1 PE=1 SV=2 [MASS=37654]	IGHA1_HUMAN	38 kDa		19	32
6 Ig lambda chain C regions OS=Homo sapiens GN=IGLC1 PE=1 SV=1 [MASS=11236]	LAC_HUMAN	11 kDa		14	30
7 Alpha-1-antitrypsin OS=Homo sapiens GN=SERPINA1 PE=1 SV=3 [MASS=46736]	A1AT_HUMAN	47 kDa		4	31
8 Complement C3 OS=Homo sapiens GN=C3 PE=1 SV=2 [MASS=187147]	CO3_HUMAN	187 kDa			26
9 Polymeric immunoglobulin receptor OS=Homo sapiens GN=PIGR PE=1 SV=4 [MASS=83283]	PIGR_HUMAN	83 kDa		30	6
10 Hemopexin OS=Homo sapiens GN=HPX PE=1 SV=2 [MASS=51676]	HEMO_HUMAN	52 kDa	TRUE	10	14
11 Alpha-1-acid glycoprotein 1 OS=Homo sapiens GN=ORM1 PE=1 SV=1 [MASS=23511]	A1AG1_HUMAN	24 kDa	TRUE	11	11
12 Haptoglobin OS=Homo sapiens GN=HP PE=1 SV=1 [MASS=45205]	HPT_HUMAN	45 kDa		3	16
13 Apolipoprotein A-I OS=Homo sapiens GN=APOA1 PE=1 SV=1 [MASS=30778]	APOA1_HUMAN	31 kDa			18
14 Ig gamma-2 chain C region OS=Homo sapiens GN=IGHG2 PE=1 SV=2 [MASS=35900]	IGHG2_HUMAN	36 kDa	TRUE		9
15 Vitamin D-binding protein OS=Homo sapiens GN=GC PE=1 SV=1 [MASS=52963]	VTDB_HUMAN	53 kDa		11	5
16 Alpha-2-macroglobulin OS=Homo sapiens GN=A2M PE=1 SV=1 [MASS=163277]	A2MG_HUMAN	163 kDa	TRUE		7
17 Ig mu chain C region OS=Homo sapiens GN=IGHM PE=1 SV=3 [MASS=49306]	IGHM_HUMAN	49 kDa			10
18 Ig gamma-3 chain C region OS=Homo sapiens GN=IGHG3 PE=1 SV=2 [MASS=41287]	IGHG3_HUMAN	41 kDa	TRUE	7	6
19 Lysozyme C OS=Homo sapiens GN=LYZ PE=1 SV=1 [MASS=16537]	LYSC_HUMAN	17 kDa		9	
20 CD44 antigen OS=Homo sapiens GN=CD44 PE=1 SV=2 [MASS=81553]	CD44_HUMAN	82 kDa		8	
21 Alpha-1B-glycoprotein OS=Homo sapiens GN=A1BG PE=1 SV=3 [MASS=54272]	A1BG_HUMAN	54 kDa		3	2
22 Ig kappa chain V-III region GOL OS=Homo sapiens PE=1 SV=1 [MASS=11830]	KV307_HUMAN (+5)	12 kDa		2	6
23 Transthyretin OS=Homo sapiens GN=TTR PE=1 SV=1 [MASS=15887]	TTHY_HUMAN	16 kDa		8	
24 Zinc-alpha-2-glycoprotein OS=Homo sapiens GN=AZGP1 PE=1 SV=1 [MASS=33872]	ZA2G_HUMAN	34 kDa		8	
25 Beta-2-microglobulin OS=Homo sapiens GN=B2M PE=1 SV=1 [MASS=13714]	B2MG_HUMAN	14 kDa		7	
26 Alpha-1-antichymotrypsin OS=Homo sapiens GN=SERPINA3 PE=1 SV=2 [MASS=47651]	AACT_HUMAN	48 kDa		2	5
27 Ceruloplasmin OS=Homo sapiens GN=CP PE=1 SV=1 [MASS=122204]	CERU_HUMAN	122 kDa			5
28 Apolipoprotein A-II OS=Homo sapiens GN=APOA2 PE=1 SV=1 [MASS=11175]	APOA2_HUMAN	11 kDa			5
29 Ig heavy chain V-III region BRO OS=Homo sapiens PE=1 SV=1 [MASS=13227]	HV305_HUMAN (+1)	13 kDa			6
30 Beta-2-glycoprotein 1 OS=Homo sapiens GN=APOH PE=1 SV=3 [MASS=38298]	APOH_HUMAN	38 kDa		3	
31 Immunoglobulin J chain OS=Homo sapiens GN=IGJ PE=1 SV=3 [MASS=15594]	IGJ_HUMAN	16 kDa			5
32 Ig alpha-2 chain C region OS=Homo sapiens GN=IGHA2 PE=1 SV=3 [MASS=36526]	IGHA2_HUMAN	37 kDa			4
33 Hemoglobin subunit beta OS=Homo sapiens GN=HBB PE=1 SV=2 [MASS=15998]	HBB_HUMAN (+4)	16 kDa			4
34 Ig kappa chain V-III region SIE OS=Homo sapiens PE=1 SV=1 [MASS=11775]	KV302_HUMAN (+3)	12 kDa	TRUE		
35 Annexin A5 OS=Homo sapiens GN=ANXA5 PE=1 SV=2 [MASS=35937]	ANXA5_HUMAN	36 kDa			2
36 Intercellular adhesion molecule 1 OS=Homo sapiens GN=ICAM1 PE=1 SV=2 [MASS=57825]	ICAM1_HUMAN	58 kDa		4	
37 Galectin-3-binding protein OS=Homo sapiens GN=LGALS3BP PE=1 SV=1 [MASS=65331]	LG3BP_HUMAN	65 kDa		4	
38 Neutrophil gelatinase-associated lipocalin OS=Homo sapiens GN=LCN2 PE=1 SV=2 [MASS=22588]	NGAL_HUMAN	23 kDa		4	
39 Pulmonary surfactant-associated protein A2 OS=Homo sapiens GN=SFTPA2 PE=1 SV=1 [MASS=26182]	SFPA2_HUMAN (+1)	26 kDa		4	
40 Ig kappa chain V-IV region Len OS=Homo sapiens PE=1 SV=2 [MASS=12640]	KV402_HUMAN	13 kDa			2
41 Antithrombin-III OS=Homo sapiens GN=SERPINC1 PE=1 SV=1 [MASS=52602]	ANT3_HUMAN	53 kDa			3
42 Epididymal secretory protein E1 OS=Homo sapiens GN=NPC2 PE=1 SV=1 [MASS=16570]	NPC2_HUMAN	17 kDa		3	
43 Pulmonary surfactant-associated protein B OS=Homo sapiens GN=SFTPB PE=1 SV=3 [MASS=42117]	PSPB_HUMAN	42 kDa			3
44 Ig heavy chain V-III region TIL OS=Homo sapiens PE=1 SV=1 [MASS=12356]	HV304_HUMAN (+1)	12 kDa	TRUE		3
45 Clusterin OS=Homo sapiens GN=CLU PE=1 SV=1 [MASS=52494]	CLUS_HUMAN	52 kDa			2
46 Cystatin-B OS=Homo sapiens GN=CSTB PE=1 SV=2 [MASS=11140]	CYTB_HUMAN	11 kDa		2	
47 Fibrinogen beta chain OS=Homo sapiens GN=FGB PE=1 SV=2 [MASS=55928]	FIBB_HUMAN	56 kDa	TRUE		2
48 Ig lambda chain V-III region SH OS=Homo sapiens PE=1 SV=1 [MASS=11392]	LV301_HUMAN	11 kDa			2
49 Ig lambda chain V-III region LOI OS=Homo sapiens PE=1 SV=1 [MASS=11935]	LV302_HUMAN	12 kDa			2

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Table 2. Proteomic screen of CM from rat AEC2 treated 24h ± 50 ng/ml Annexin V, undepleted of surfactant

Protein Name	Accession Number	MW	Protein Grouping		AEC CM	
			Ambiguity	control	Annexin V	
1 L-lactate dehydrogenase A chain OS=Rattus norvegicus GN=Ldha PE=1 SV=1 [MASS=36450]	LDHA_RAT	36 kDa			12	8
2 Moesin OS=Rattus norvegicus GN=Msn PE=1 SV=3 [MASS=67738]	MOES_RAT	68 kDa			16	7
3 Uteroglobin OS=Rattus norvegicus GN=Scgb1a1 PE=1 SV=2 [MASS=10449]	UTER_RAT	10 kDa			6	11
4 Actin, cytoplasmic 2 OS=Rattus norvegicus GN=Actg1 PE=1 SV=1 [MASS=41793]	ACTG_RAT (+1)	42 kDa			12	11
5 Cathepsin B OS=Rattus norvegicus GN=Ctsb PE=1 SV=2 [MASS=37470]	CATB_RAT	37 kDa			11	7
6 Connective tissue growth factor OS=Rattus norvegicus GN=Ctgf PE=2 SV=1 [MASS=37756]	CTGF_RAT	38 kDa			7	10
7 Plasminogen activator inhibitor 1 OS=Rattus norvegicus GN=Serpine1 PE=2 SV=1 [MASS=45009]	PAI1_RAT	45 kDa			10	4
8 SPARC OS=Rattus norvegicus GN=Sparc PE=1 SV=4 [MASS=34296]	SPRC_RAT	34 kDa			8	5
9 Clusterin OS=Rattus norvegicus GN=Clu PE=1 SV=2 [MASS=51375]	CLUS_RAT	51 kDa			5	8
10 Ubiquitin OS=Rattus norvegicus GN=Rps27a PE=1 SV=1 [MASS=8565]	UBIQ_RAT	9 kDa			7	5
11 Peroxiredoxin-6 OS=Rattus norvegicus GN=Prdx6 PE=1 SV=3 [MASS=24818]	PRDX6_RAT	25 kDa			7	5
12 Histone H2A.J OS=Rattus norvegicus GN=H2afj PE=2 SV=1 [MASS=14045]	H2AJ_RAT (+6)	14 kDa			6	5
13 Anionic trypsin-1 OS=Rattus norvegicus GN=Prss1 PE=1 SV=1 [MASS=25959]	TRY1_RAT	26 kDa			5	
14 14-3-3 protein zeta/delta OS=Rattus norvegicus GN=Ywhaz PE=1 SV=1 [MASS=27771]	1433Z_RAT	28 kDa			6	3
15 Fructose-bisphosphate aldolase A OS=Rattus norvegicus GN=Aldoa PE=1 SV=2 [MASS=39352]	ALDOA_RAT	39 kDa			2	5
16 Heat shock cognate 71 kDa protein OS=Rattus norvegicus GN=Hspa8 PE=1 SV=1 [MASS=70871]	HSP7C_RAT	71 kDa			4	
17 Cystatin-C OS=Rattus norvegicus GN=Cst3 PE=1 SV=2 [MASS=15437]	CYTC_RAT	15 kDa			5	3
18 14-3-3 protein epsilon OS=Rattus norvegicus GN=Ywhae PE=1 SV=1 [MASS=29174]	1433E_RAT	29 kDa			5	2
19 Serum albumin OS=Rattus norvegicus GN=Alb PE=1 SV=2 [MASS=68730]	ALBU_RAT	69 kDa			3	
20 Alpha-actinin-1 OS=Rattus norvegicus GN=Actn1 PE=1 SV=1 [MASS=102960]	ACTN1_RAT (+1)	103 kDa			3	2
21 1-phosphatidylinositol-4,5-bisphosphate phosphodiesterase epsilon-1 OS=Rattus norvegicus GN=Plce1 PE=1 SV=1 [MASS=255391]	PLCE1_RAT	255 kDa				2
22 Voltage-dependent N-type calcium channel subunit alpha-1B OS=Rattus norvegicus GN=Cacna1b PE=1 SV=1 [MASS=262254]	CAC1B_RAT (+1)	262 kDa			2	
23 Lysozyme C-1 OS=Rattus norvegicus GN=Lyz1 PE=1 SV=2 [MASS=16729]	LYSC1_RAT (+1)	17 kDa				2
24 14-3-3 protein gamma OS=Rattus norvegicus GN=Ywhag PE=1 SV=2 [MASS=28302]	1433G_RAT	28 kDa			2	2
25 Profilin-1 OS=Rattus norvegicus GN=Pfn1 PE=1 SV=2 [MASS=14957]	PROF1_RAT	15 kDa			2	2
26 Serum response factor-binding protein 1 OS=Rattus norvegicus GN=Srfbp1 PE=2 SV=1 [MASS=49195]	SRFB1_RAT	49 kDa			3	2
27 SEC14-like protein 3 OS=Rattus norvegicus GN=Sec14l3 PE=1 SV=1 [MASS=46027]	S14L3_RAT	46 kDa				2
28 Histone H2B type 1-A OS=Rattus norvegicus GN=Hist1h2ba PE=1 SV=2 [MASS=14224]	H2B1A_RAT (+1)	14 kDa			3	
29 Secreted frizzled-related protein 1 (Fragment) OS=Rattus norvegicus GN=Sfrp1 PE=2 SV=1 [MASS=18072]	SFRP1_RAT	18 kDa				3
30 Histone H3.1 OS=Rattus norvegicus PE=1 SV=3 [MASS=15404]	H31_RAT (+1)	15 kDa			5	
31 Leucine-rich repeat-containing protein 63 OS=Rattus norvegicus GN=Lrrc63 PE=2 SV=1 [MASS=69216]	LRC63_RAT	69 kDa			5	
32 Heat shock protein HSP 90-alpha OS=Rattus norvegicus GN=Hsp90aa1 PE=1 SV=3 [MASS=84814]	H590A_RAT (+1)	85 kDa			2	2
33 Lactadherin OS=Rattus norvegicus GN=Mfge8 PE=2 SV=1 [MASS=47413]	MFGM_RAT	47 kDa			2	
34 Tropomyosin alpha-3 chain OS=Rattus norvegicus GN=Tpm3 PE=1 SV=2 [MASS=29006]	TPM3_RAT	29 kDa			2	
35 Carbonic anhydrase 2 OS=Rattus norvegicus GN=Ca2 PE=1 SV=2 [MASS=29114]	CAH2_RAT	29 kDa			2	
36 Peptidyl-prolyl cis-trans isomerase A OS=Rattus norvegicus GN=Ppia PE=1 SV=2 [MASS=17874]	PPIA_RAT	18 kDa			3	
37 Transaldolase OS=Rattus norvegicus GN=Taldo1 PE=1 SV=2 [MASS=37460]	TALDO_RAT	37 kDa			4	
38 Histone H1.2 OS=Rattus norvegicus GN=Hist1h1c PE=1 SV=3 [MASS=21987]	H12_RAT	22 kDa			4	
39 Microtubule-associated protein 1B OS=Rattus norvegicus GN=Map1b PE=1 SV=2 [MASS=269498]	MAP1B_RAT	270 kDa				2
40 Carboxylesterase 3 OS=Rattus norvegicus GN=Ces3 PE=1 SV=2 [MASS=62147]	CES3_RAT	62 kDa			2	
41 Eukaryotic translation initiation factor 5B OS=Rattus norvegicus GN=Eif5b PE=1 SV=1 [MASS=137685]	IF2P_RAT	138 kDa				2
42 Malate dehydrogenase, mitochondrial OS=Rattus norvegicus GN=Mdh2 PE=1 SV=2 [MASS=35683]	MDHM_RAT	36 kDa			2	
43 Superoxide dismutase [Cu-Zn] OS=Rattus norvegicus GN=Sod1 PE=1 SV=2 [MASS=15912]	SODC_RAT	16 kDa			2	

Note: There is large amount of surfactant present in AEC2 CM which can obscure some proteins, so the screen was repeated after detergent depletion- see Table 3.

Table 3. Proteomic screen of surfactant-depleted CM from rat AEC2 treated 24h ± 50 ng/ml Annexin

V

Protein Name	Accession Number	MW	Protein Grouping Ambiguity	AEC CM	
				control	Annexin V
1 L-lactate dehydrogenase A chain OS=Rattus norvegicus GN=Ldha PE=1 SV=1 [MASS=36450]	LDHA_RAT	36 kDa		15	14
2 Uteroglobin OS=Rattus norvegicus GN=Scgb1a1 PE=1 SV=2 [MASS=10449]	UTER_RAT	10 kDa		11	18
3 Actin, cytoplasmic 2 OS=Rattus norvegicus GN=Actg1 PE=1 SV=1 [MASS=41793]	ACTG_RAT (+1	42 kDa		14	13
4 Connective tissue growth factor OS=Rattus norvegicus GN=Ctgf PE=2 SV=1 [MASS=37756]	CTGF_RAT	38 kDa		15	14
5 Serum albumin OS=Rattus norvegicus GN=Alb PE=1 SV=2 [MASS=68730]	ALBU_RAT	69 kDa		4	5
6 Moesin OS=Rattus norvegicus GN=Msn PE=1 SV=3 [MASS=67738]	MOES_RAT	68 kDa	TRUE	14	7
7 Cathepsin B OS=Rattus norvegicus GN=Ctsb PE=1 SV=2 [MASS=37470]	CATB_RAT	37 kDa	TRUE	12	8
8 Clusterin OS=Rattus norvegicus GN=Clu PE=1 SV=2 [MASS=51375]	CLUS_RAT	51 kDa		6	11
9 Peroxiredoxin-6 OS=Rattus norvegicus GN=Prdx6 PE=1 SV=3 [MASS=24818]	PRDX6_RAT	25 kDa		10	4
10 Plasminogen activator inhibitor 1 OS=Rattus norvegicus GN=Serpine1 PE=2 SV=1 [MASS=45009]	PAI1_RAT	45 kDa		7	10
11 Heat shock cognate 71 kDa protein OS=Rattus norvegicus GN=Hspa8 PE=1 SV=1 [MASS=70871]	HSP7C_RAT	71 kDa	TRUE	9	5
12 14-3-3 protein zeta/delta OS=Rattus norvegicus GN=Ywhaz PE=1 SV=1 [MASS=27771]	1433Z_RAT	28 kDa		9	4
13 SPARC OS=Rattus norvegicus GN=Sparc PE=1 SV=4 [MASS=34296]	SPRC_RAT	34 kDa		6	6
14 SEC14-like protein 3 OS=Rattus norvegicus GN=Sec14l3 PE=1 SV=1 [MASS=46027]	S14L3_RAT	46 kDa		8	2
15 Histone H4 OS=Rattus norvegicus GN=Hist1h4b PE=1 SV=2 [MASS=11367]	H4_RAT	11 kDa		6	2
16 Ubiquitin OS=Rattus norvegicus GN=Rps27a PE=1 SV=1 [MASS=8565]	UBIQ_RAT	9 kDa	TRUE	4	3
17 Peptidyl-prolyl cis-trans isomerase A OS=Rattus norvegicus GN=Ppia PE=1 SV=2 [MASS=17874]	PPIA_RAT	18 kDa		6	3
18 Histone H2B type 1-A OS=Rattus norvegicus GN=Hist1h2ba PE=1 SV=2 [MASS=14224]	H2B1A_RAT (+	14 kDa		3	2
19 Superoxide dismutase [Cu-Zn] OS=Rattus norvegicus GN=Sod1 PE=1 SV=2 [MASS=15912]	SODC_RAT	16 kDa		3	3
20 Histone H2A.J OS=Rattus norvegicus GN=H2afj PE=2 SV=1 [MASS=14045]	H2AJ_RAT (+7	14 kDa		3	3
21 WAP four-disulfide core domain protein 2 OS=Rattus norvegicus GN=Wfdc2 PE=2 SV=1 [MASS=17448]	WFDC2_RAT	17 kDa		4	3
22 Rab GDP dissociation inhibitor beta OS=Rattus norvegicus GN=Gdi2 PE=1 SV=2 [MASS=50537]	GDIB_RAT	51 kDa	TRUE	5	2
23 14-3-3 protein epsilon OS=Rattus norvegicus GN=Ywhae PE=1 SV=1 [MASS=29174]	1433E_RAT	29 kDa	TRUE	5	3
24 Annexin A5 OS=Rattus norvegicus GN=Anxa5 PE=1 SV=3 [MASS=35744]	ANXA5_RAT	36 kDa			6
25 Hemoglobin subunit beta-1 OS=Rattus norvegicus GN=Hbb PE=1 SV=3 [MASS=15979]	HBB1_RAT (+1	16 kDa		4	2
26 Alpha-actinin-4 OS=Rattus norvegicus GN=Actn4 PE=1 SV=2 [MASS=104914]	ACTN4_RAT (+	105 kDa		4	
27 Cystatin-C OS=Rattus norvegicus GN=Cst3 PE=1 SV=2 [MASS=15437]	CYTC_RAT	15 kDa		4	2
28 Profilin-1 OS=Rattus norvegicus GN=Pfn1 PE=1 SV=2 [MASS=14957]	PROF1_RAT	15 kDa		4	
29 Protein S100-G OS=Rattus norvegicus GN=S100g PE=1 SV=3 [MASS=9038]	S100G_RAT	9 kDa		4	
30 Selenium-binding protein 1 OS=Rattus norvegicus GN=Selenbp1 PE=1 SV=1 [MASS=52532]	SBP1_RAT	53 kDa		4	
31 Alpha-enolase OS=Rattus norvegicus GN=Eno1 PE=1 SV=4 [MASS=47128]	ENOA_RAT	47 kDa		3	
32 Thioredoxin OS=Rattus norvegicus GN=Txn PE=1 SV=2 [MASS=11673]	THIO_RAT	12 kDa		2	2
33 Protein disulfide-isomerase A3 OS=Rattus norvegicus GN=Pdia3 PE=1 SV=2 [MASS=56623]	PDIA3_RAT	57 kDa	TRUE		2
34 Heat shock protein HSP 90-beta OS=Rattus norvegicus GN=Hsp90ab1 PE=1 SV=4 [MASS=83281]	HS90B_RAT	83 kDa			2
35 Sulfated glycoprotein 1 OS=Rattus norvegicus GN=Psap PE=1 SV=1 [MASS=61124]	SAP_RAT	61 kDa		3	
36 Transaldolase OS=Rattus norvegicus GN=Taldo1 PE=1 SV=2 [MASS=37460]	TALDO_RAT	37 kDa		3	
37 Malate dehydrogenase, cytoplasmic OS=Rattus norvegicus GN=Mdh1 PE=1 SV=3 [MASS=36483]	MDHC_RAT	36 kDa		3	
38 Protein S100-A11 OS=Rattus norvegicus GN=S100a11 PE=3 SV=1 [MASS=11065]	S10AB_RAT	11 kDa			2
39 Fructose-bisphosphate aldolase A OS=Rattus norvegicus GN=Aldoa PE=1 SV=2 [MASS=39352]	ALDOA_RAT	39 kDa		2	
40 Rho GDP-dissociation inhibitor 1 OS=Rattus norvegicus GN=Arhgdia PE=1 SV=1 [MASS=23407]	GDIR1_RAT	23 kDa		2	
41 Keratin, type II cytoskeletal 1 OS=Rattus norvegicus GN=Krt1 PE=2 SV=1 [MASS=64830]	K2C1_RAT (+2	65 kDa		2	
42 Lactadherin OS=Rattus norvegicus GN=Mfge8 PE=2 SV=1 [MASS=47413]	MFGM_RAT	47 kDa		2	
43 Phosphatidylethanolamine-binding protein 1 OS=Rattus norvegicus GN=Pebp1 PE=1 SV=3 [MASS=20801]	PEBP1_RAT	21 kDa			2
44 High mobility group protein B1 OS=Rattus norvegicus GN=Hmgb1 PE=1 SV=2 [MASS=24894]	HMGB1_RAT	25 kDa		2	
45 Aspartate aminotransferase, mitochondrial OS=Rattus norvegicus GN=Got2 PE=1 SV=2 [MASS=47314]	AATM_RAT	47 kDa		2	
46 Dihydropyrimidinase-related protein 2 OS=Rattus norvegicus GN=Dpysl2 PE=1 SV=1 [MASS=62277]	DPYL2_RAT	62kD			
47 Malate dehydrogenase, mitochondrial OS=Rattus norvegicus GN=Mdh2 PE=1 SV=2 [MASS=35683]	MDHM_RAT	36 kDa		2	
48 Peptidyl-prolyl cis-trans isomerase B OS=Rattus norvegicus GN=PpiB PE=2 SV=3 [MASS=23802]	PPIB_RAT	24 kDa		2	

Table 4. Proteomic screen of CM from human lung fibroblast cell line CCD-8Lu after 24h \pm 50 ng/ml Annexin V

Protein Name	Accession Number	MW	Protein Grouping	
			Ambiguity	fibroblast CM
			control	Annexin V
1 Serum albumin OS=Homo sapiens GN=ALB PE=1 SV=2 [MASS=69366]	ALBU_HUMAN	69 kDa	83	108
2 Collagen alpha-1(I) chain OS=Homo sapiens GN=COL1A1 PE=1 SV=4 [MASS=138910]	CO1A1_HUMAN	139 kDa	5	23
3 Alpha-2-HS-glycoprotein OS=Homo sapiens GN=AHSG PE=1 SV=1 [MASS=39324]	FETUA_HUMAN	39 kDa	10	
4 Lactotransferrin OS=Homo sapiens GN=LTF PE=1 SV=6 [MASS=78181]	TRFL_HUMAN	78 kDa	16	3
5 Collagen alpha-2(I) chain OS=Homo sapiens GN=COL1A2 PE=1 SV=6 [MASS=129287]	CO1A2_HUMAN	129 kDa	3	8
6 Decorin OS=Homo sapiens GN=DCN PE=1 SV=1 [MASS=39746]	PGS2_HUMAN	40 kDa	2	6
7 Beta-2-microglobulin OS=Homo sapiens GN=B2M PE=1 SV=1 [MASS=13714]	B2MG_HUMAN	14 kDa	3	
8 Annexin A5 OS=Homo sapiens GN=ANXA5 PE=1 SV=2 [MASS=35937]	ANXA5_HUMAN	36 kDa		5
9 Fibronectin OS=Homo sapiens GN=FN1 PE=1 SV=3 [MASS=262605]	FINC_HUMAN	263 kDa		4
10 POTE ankyrin domain family member F OS=Homo sapiens GN=POTEF PE=1 SV=2 [MASS=121444]	POTEF_HUMAN (+7)	121 kDa		4
11 Sulfhydryl oxidase 1 OS=Homo sapiens GN=QSOX1 PE=1 SV=3 [MASS=82577]	QSOX1_HUMAN	83 kDa		3