A molecular signature of lung cancer: potential biomarkers for adenocarcinoma and squamous cell carcinoma

SUPPLEMENTARY MATERIALS

Materials and methods

Gel electrophoresis and immunoblotting

Samples (10-40 µg of protein) were subjected to SDS-PAGE. Gels were stained with Coommassie Brilliant Blue or electro-transferred onto nitrocellulose membranes for immunostaining. Membranes containing the transferred proteins were blocked with 5% non-fat dry milk and 0.1% Tween-20 in Tris-buffered saline (TBS) and incubated overnight at 4°C with the different primary antibodies (sources and dilutions as detailed in Supplementary Table 2), followed by incubation with the appropriate HRP-conjugated secondary antibodies for 1 h. Enhanced chemiluminescence (Biological Industries) was used for detection of HRP activity. Band intensities were analyzed using FUSION-FX (Vilber Lourmat, France) and the values were normalized to the intensities of the appropriate α-actin signal that served as a loading control.

RNA isolation and q-PCR

Total RNA was isolated from healthy and tumor lung samples using an RNeasy mini kit (Qiagen) according to the manufacturer's instructions. Total RNA quality was analyzed using the Agilent RNA 6000 nano kit. q-PCR was performed using specific primers (KiCqStart Primers; Sigma Aldrich) in triplicate, using Power SYBER green master mix (Applied Biosystems, Foster City, CA). Levels of target genes were normalized relative to β-actin mRNA levels. Samples were amplified by a 7300 Real Time PCR System (Applied Biosystems) for 40 cycles using the following PCR parameters: 95°C for 15 seconds, 60°C for 1 minute, and 72°C for 1 minute. The copy numbers for each sample were calculated by the CT-based calibrated standard curve method. The mean fold changes (± SEM) of the three replicates were calculated. Genes examined and primers used are listed in Supplementary Table 4.

LC-HR MS/MS analysis

Samples were subjected to in-solution tryptic digestion as follows. Proteins were first reduced by incubation with 5 mM DTT for 30 min at 60°C, followed by alkylation with 10 mM iodoacetamide in the dark for 30 min at 21°C. Proteins were then subjected to digestion with trypsin (Promega, Madison, WI) at a 1:50 trypsin:protein ratio for 16 h at 37°C. Following digestion, detergents were cleared from the samples using commercial detergent removal columns (Pierce, Rockford, IL), and desalted using solid-phase extraction columns (Oasis HLB, Waters, Milford, MA). Digestions were stopped by addition of trifluroacetic acid (1%). The samples were stored at -80°C until LC-HR MS/MS analysis.

For LC-HR MS/MS, ULC/MS grade solvents were used for all chromatographic steps. Each sample was separated using split-less nano-ultra performance liquid chromatography columns (10 kpsi nanoAcquity; Waters). The mobile phase was (A) H_2O and 0.1% formic acid, and (B) acetonitrile and 0.1% formic acid. Desalting of the samples was performed online using a reverse-phase C18 trapping column (180 µm internal diameter, 20 mm length, 5 µm particle size; Waters). The peptides were then separated using a T3 HSS nano-column (75 µm internal diameter, 250 mm length, 1.8 µm particle size; Waters) at 0.3 µL/min. Peptides were eluted from the column into the mass spectrometer using the following gradient: 4% to 35% (B) for 150 min, 35% to 90% (B) for 5 min, maintained at 90% for 5 min and then back to initial conditions. The nano-UPLC was coupled online through a nano-ESI emitter (10 µm tip; New Objective, Woburn, MA) to a quadrupole Orbitrap mass spectrometer (Q Executive, Thermo Scientific) using a FlexIon nanospray apparatus (Proxeon). Data was acquired in the DDA mode, using a Top12 method [1]. Raw data was imported into Expressionist software (Genedata) [2, 3], The software was used for retention time alignment and peak detection of precursor peptide intensities. A master peak list was generated from all MS/MS events and sent for database searching using Mascot v2.4 (Matrix Sciences). Data was searched against a database containing forward and reverse human protein sequences from UniprotKB/SwissProt, and 125 common laboratory contaminants, totaling 20,304 entries. Fixed modification was set to carbamidomethylation of cysteines, while variable modification was set to oxidation of methionines. Search results were then imported back to Expressionist for annotation of detected peaks. Identifications were filtered such that the global false discovery rate was a maximum of 1%. Protein abundance was calculated based on the three most abundant peptides [4].

Proteins with less than 2 unique peptides were excluded from further analysis.

Samples from 9 patients were analyzed, with healthy and cancerous lung tissues being taken from the

same patient lung. Proteins for which at least two unique peptides were identified were used for further analysis.

Immunohistochemistry (IHC) on tissue microarray (TMA) slides

Immunohistochemical staining was performed on formalin-fixed and paraffin-embedded tissue microarray slides obtained from Biomax US. The sections were deparaffinized using xylene and a graded ethanol series. Endogenous peroxidase activity was blocked by incubating the sections in 3% H_2O_2 for 10 minutes. Antigen retrieval was performed in 0.01M citrate buffer (pH 6.0) at 95°C-98°C for 20 min. After washing sections in PBS (pH 7.4), non-specific antibody binding was reduced by incubating the sections in 10% normal goat serum for 2 h. After decanting excess serum, sections were incubated overnight at 4°C with primary antibodies (Supplementary Table 2). After washing with PBS, the sections were incubated for 2 h with the appropriate secondary antibodies conjugated to horseradish peroxidase (Supplementary Table 2). Sections were washed three times in PBS and subsequently, the peroxidase-catalyzed reaction was visualized by incubating with 0.02% DAB. After rinsing in water, the sections were counterstained with hematoxylin, and mounted with Vectashield mounting medium (Vector Laboratories, Burlingame, CA). Finally, the sections were observed under a microscope (DM2500, Leica) and images were taken at the indicated magnification with the same light intensity and exposure time. Controls were carried out with the same protocols but omitting the primary antibodies.

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Supplementary Table 1: Summary of current reported or used in the clinic biomarkers for AC and SCC

No	NSCLC reported markers	Marker for: (Ref)
1.	miR21	AC [5]
2.	EGFR - Epidermal growth factor receptor (tyrosine kinase)	Over-expressed in NSCLC [6, 7], AC [8]
3.	ALK-EML4- Tyrosine-protein kinase receptor	AC [9, 10]
4.	ROS1- Proto-oncogene tyrosine-protein kinase ROS	AC [11]
5.	RET- Proto-oncogene tyrosine-protein kinase receptor Ret	AC [12]
6.	c-MET -Hepatocyte growth factor receptor (tyrosine kinase)	Over-expressed in NSCLC [13, 14]
7.	ERBB2- Receptor tyrosine-protein kinase erbB-2	AC [15]
8.	PPP3CA- Serine/threonine-protein phosphatase 2B catalytic subunit alpha isoform. (Mutation)	AC [16]
9.	DOT1L- Histone-lysine N-methyltransferase, H3 lysine-79 specific. (Mutated)	AC [17]
10.	FTSJD1- cap-specific mRNA (nucleoside-2'-O-)-methyltransferase 2. (Mutation)	AC [17]
11.	TTF1-thyroid transcription factor 1	AC [18]
12.	NAPSA- Napsin A	AC [18]
13.	TP63- Tumor protein 63	SCC [19]
14.	p40- ΔNp63	SCC [18, 20]
15.	RASA1- Ras GTPase-activating protein 1	SCC [6, 7]
16.	CD141- Thrombomodulin	SCC [21, 22]
17.	miR205	SCC [5]

The biomarkers used in the clinic are highlighted in grey color. Markers 2 to 6 are predictive marker used to direct targeted therapy and markers 11-14 serve in the clinic for diagnosis of AC or SCC.

Supplementary Table 2: Antibodies used in this study

Antibody	Source and Catalogue Number	Dilution used	
		IHC	WB
Mouse monoclonal anti- β-Actin	Millipore, Billerica, MA, MAB1501	-	1:10,000
Mouse monoclonal anti-ATP5B	Abcam, Cambridge, UK, ab14730	-	1:10,000
Rabbit monoclonal anti-AIF	Abcam, ab32516	1:200	1:1000
Mouse monoclonal anti-Bcl-2	Calbiochem, Nottingham, UK, OP60	-	1:2000
Mouse monoclonal anti-HK-I	Abcam, ab105213	1:500	1:2000
Rabbit monoclonal anti-HK II	Santa Cruz Biotechnology Dallas, TX, sc-27230	-	1:1000
Goat polyclonal anti-LDHA	Epitomics, Cambridge, UK, 1980-1	1:300	1:1000
Rabbit polyclonal anti-MAVS	ALX-210-929-C100		1:2000
Rabbit monoclonal anti-HYOU1	Abcam, ab134944		1:3000
Rabbit monoclonal anti-Hsp60	Abcam, ab46798	-	1:10,000
Mouse monoclonal anti -GAPDH	Abcam, ab9484	-	1:1000
Rabbit monoclonal anti-Rab11b	Santa Cruz Biotechnology, Dallas, TX, ab3612	-	1:1000
Rabbit monoclonal anti-SMAC/ Diablo	Abcam, ab8115	1:300	1:2000
Rabbit monoclonal anti-VDAC1	Abcam, ab15895	1:500	1:5000
Goat anti-Rabbit-HRP	KPL, Gaithersburg, PA, 474-1506	1:250	1:15,000
Donkey anti-Goat-HRP	Abcam, ab97120	-	1:20,000

Antibodies against the indicated protein, their catalogue number, source and the dilutions used in IHC and immunoblot experiments are presented.

Supplementary Table 3: Lung cancer patient characteristics

Patient No.	Age (years)	Gender	Type of Cancer	Stage of Disease
1	76	F	AC	2B
2	77	M	SCC	1A
3	54	M	AC	3A
4	58	M	SCC	2B
5	70	M	SCC	2B
6	69	F	AC	2A
7	36	M	AC	3A
8	62	M	AC	1A
9	82	M	AC	2A
10	48	M	AC	1A
11	65	F	AC	1A
12	78	M	SCC	1B
13	72	M	AC	1A
14	78	M	AC	1A
15	55	M	AC	1A
16	59	F	SCC	1A
17	74	F	SCC	2A
18	65	M	SCC	4
19	76	M	LCC	3A
20	65	F	SCC	1A
21	61	M	AC	1A
22	54	M	SCC	1B
23	56	F	AC	1A
24	58	M	AC	1A
25	55	F	AC	1A
26	76	F	AC	1A
27	85	M	AC	1B
28	55	F	AC	1A
29	62	M	AC	2A
30	79	M	AC	3A
31	81	M	AC	1A
32	62	F	AC	1B
33	83	F	SCC	1B
34	77	M	SCC	1A
35	86	M	SCC	1B
36	74	M	SCC	1B
37	74	M	AC	1A
38	67	F	AC	2A
39	71	M	AC	1A
40	75	F	AC	3A
41	85	M	SCC	1A
42	77	M	SCC	2A
43	59	F	SCC	2A
44	59	F	SCC	1B
45	58	M	AC	1B
46	68	F	AC	1A

Twenty-eight patients were males and twenty-seven were females, with an average age of 68 years (range, 36-86). Disease stage was staged according to the international tumor-node-metastasis system and classified as well (grade 1, n=30), (grade 2, n=10), (grade 3, n=5) (grade 4, n=1).

Supplementary Table 4: qPCR primers used in this study

Gene	Primer sequences
ß-Actin	Forward 5'-ACTCTTCCAGCCTTCCTTCC-3' Reverse 5'- TGTTGGCGTACAGGTCTTTG-3'
AKR1B10	Forward 5'-GAGCAGGACGTGAGACTTCT-3' Reverse 5'-TTTGCCAAGAGGAGACTTCCAA-3'
USP14	Forward 5'-TGCCCTTAAAAGGTATGCAGGT-3' Reverse 5'- TCTCGGCAAACTGTGGGAAA-3'
TTLL12	Forward 5'-TGGAGCACGAGGTTTTCGAC-3' Reverse 5'- CGATGACCTTGTAGCACAGC-3'
TSG101	Forward 5'-GCCAGCTCAAGAAAATGGTGT-3' Reverse 5'- AGGTCTCTGTATTTGTACTGGGT-3'
LRRF2	Forward 5'-CCTCAGCAACAACCCCTCTA-3' Reverse 5'- GGTCATAGATATCCCGCAATTCA-3'
WDR82	Forward 5'-GCTTCGATTTCAGCCCCAAC-3' Reverse 5'- TCTCTTTGGTTTGCCCTCCT-3'
HAT1	Forward 5'-ATGGCGGGATTTGGTGCTAT-3' Reverse 5'- GTTCAATTGCTGTGTTGGTGT-3'

The genes examined, and the forward and reverse sequences of the primers used are indicated.

Supplementary Table 5: Novel bio-markers for selected proteins differentially expressed in healthy donors and lung cancer patients identified by LC-HR MS/MS

See Supplementary File 1

Supplementary Table 6: Differential expression of proteins in AC and SCC

Protein (UniProt accession)	Fold change SCC/AC P-value	Proposed function (cell localization)	Relation to cancer
ITGA7 - Integrin alpha-7 (Q13683)	107.3 $1.3x10^{-3}$	Laminin receptor on skeletal myoblasts (Plasma membrane)	Associated with the occurrence and development of bladder cancer [105]
AKR1B10 - Aldo-keto reductase family 1 member B10 (O60218)	17.9 $1.9x10^{-3}$	Regulates the balance of retinoic acid and lipid metabolism (lysosome, Secreted)	Potential diagnostic marker specific to smokers' NSCLCs [106]
USP14 - Ubiquitin carboxyl-terminal hydrolase 14 (P54578)	4.4 $2.0x10^{-3}$	Proteasome-associated deubiquitinase (Cytosol, Plasma membrane)	Over-expressed in various types of cancer including NSCLC [107]
TTL12 - Tubulin-tyrosine ligase-like protein 12 (Q14166)	8.1 $2.1x10^{-3}$	Catalyze post-translational modification of tubulins (Cytosol)	Expression increases during prostate cancer progression to metastasis [108]
TSG101 - Tumor susceptibility gene 101 protein (Q99816)	-38.3 $2.6x10^{-3}$	Regulator of vesicular trafficking process (Plasma membrane, Cytosol, Nucleus)	TSG101 splicing variant is linked to progressive tumor-stage and metastasis [109]
LRRFIP2 - Leucine-rich repeat flightless-interacting protein 2 (Q9Y608)	19.3 $2.9x10^{-3}$	Positive regulator of the Toll-like receptor (TLR) signaling (Cytosol)	No reported data
WDR82 - WD repeat-containing protein 82 (Q6UXN9)	9.8 $3.1x10^{-3}$	Component of histone methyl-transferase complex (Nucleus)	No reported data
HAT1 - Histone acetyltransferase type B catalytic subunit (O14929)	475 $4.4x10^{-3}$	Acetylates soluble histone H4, a type B HAT that functions in DNA repair (Nucleus)	High expression in several types of lymphomas. proposed indicator for poor prognosis [110] and potential drug target in esophageal SCC [111]
ACOT1 - Acyl-co-enzyme A thioesterase 1 (Q86TX2)	-2.8 $5.6x10^{-3}$	Long chain fatty acid metabolism (Cytosl)	Highly expressed in luminal breast tumors [112]
RAB34 – Ras-related protein Rab-34 (Q9BZG1)	-6.0 $7.6x10^{-3}$	GTPase involved in protein transport (Cytosol, Golgi)	A progression- and prognosis-associated biomarker in gliomas [113]. Ras-associated sarcomagenesis [114]

Supplementary Table 7: Potential proteins for distinguishing between AC and SCC. Results were derived from USCS XENA data

Gene	Proposed function (cell localization)	Relation to cancer
	Higher expression levels in AC	
1. AZGP1 - Zinc-alpha-2-glycoprotein (P25311)	**Lipid degradation in adipocytes, associated with fat losses in some advanced cancers (Plasma membrane, Secreted, Exosomes)**	Associated with AC lung cancer [115]
2. ACOT1 - Acyl-coenzyme A thioesterase 1 (Q86TX2)	**Lipid metabolism, long chain fatty acid metabolism (Cytosol)**	**No reported data**
3. ACAD8 - Isobutyryl-CoA dehydrogenase (Q9UKU7)	**Acyl-CoA dehydrogenase, catabolism of valine (Mitochondria)**	**No reported data**
4. NPC2 - Epididymal secretory protein E1 (P61916)	**Cholesterol transporter (ER, Lysosome, Secreted)**	Associated with lung AC [116]
5. ABCD3 - ATP-binding cassette sub-family D member 3 (P28288)	**Involved in fatty acid transport (Peroxisome)**	Associated with lung AC [117]
6. GALE - UDP-glucose 4-epimerase (Q14376)	**Galactose metabolism (Cytosol, Exosomes)**	**No reported data**
7. FEN1 - Flap endonuclease 1 (P39748)	**Endonuclease involved in DNA replication and repair (Cytosol)**	Associated with lung AC [118]
8. AKR7A3 - Aldo-Keto Reductase family 7A isoform 3 (O95154)	**Invoved in Aflotoxin B1 inactivation (Cytosol, Exosome)**	**No reported data**
9. ARRB1 - Beta-arrestin-1 (P49407)	**Signaling pathway: Functions in regulating agonist-mediated GPCR (Membrane, Cytosol, Nucleus)**	Enhances chemosensitivity in NSCLC) [119]
10. RSU1 - Ras suppressor protein 1 (Q15404)	**Ras signal transduction pathway (Cytosol, Exosomes)**	**No reported data**
11. LRBA - Lipopoly-saccharide-responsive and beige-like anchor protein (P50851)	**Coordinates signaling of immune receptors (Cell membrane, ER, Golgi, Lysosome)**	**No reported data**
	Higher expression levels in SCC	
12. Ck5 - Keratin, type II cytoskeletal 5 (P13647)	**Structural protein (Plasma membrane, Cytosol, Nucleus, Exosome)**	Associated with lung SCC [19, 120]
13. Ck13 - Keratin, type I cytoskeletal 13 (P13646)	**Structural protein (Cytosol, Nucleus, Exosome)**	Associated with lung SCC [121]
14. Ck14 - Keratin, type I cytoskeletal 14 (P02533)	**Structural protein (Cytosol, Nucleus)**	Associated with lung SCC [120, 122]
15. Ck17 - Keratin, type I cytoskeletal 17 (Q04695)	**Structural protein (Cytosol)**	Associated with lung SCC [120]
16. PFN2 -Profilin-2 (P35080)	**Structural protein (Cytosol)**	Associated with NSCLC [123]
17.RANBP1 - Specific GTPase-activating protein (P43487)	**Signaling pathway, Inhibits GTP exchange on Ran (Cytosol, Nucleus)**	**No reported data**
18. CSTA - Cystatin-A (P01040)	**Thiol proteinase inhibitor (Cytosol, Exosomes)**	Associated with lung SCC [124, 125]
19. GGH - Gamma-glutamyl hydrolase (Q92820)	**Amino acid metabolism, Hydrolyzes polyglutamate (Lysosome, Secreted)**	Associated with lung cancer NSCLC [126]
20. RPS6KA3- Ribosomal protein S6 kinase alpha-3 (P51812)	**Serine/threonine-protein kinase acts downstream of ERK signaling (Nucleus, Cytosol)**	Associated with NSCLC [127, 128]
21. IGF2BP3-Insulin-like growth factor 2 mRNA-binding protein 3 (O00425)	**RNA-binding factor that may recruit target transcripts to cytoplasmic protein-RNA complexes (mRNPs)(Nucleus, Cytosol)**	Associated with NSCLC [129]
22. TIMM44 - Mitochondrial IMM translocase subunit TIM44 (O43615)	**Mitochondrial peptide transporter, essential component of the PAM complex, ATP binding (Mitochondria)**	**No reported data**
23. TP63 - Tumor protein 63 (Q9H3D4)	**Tumor suppressor (Nucleus)**	Associated with lung SCC [19]
24. SMC2 - Structural maintenance of chromosomes protein 2 (O95347)	Involved in **condensing chromatin complex** (Cytosol, Nucleus)	**No reported data**

Supplementary Table 8: The relationship between protein expression levels and survival in AC and SCC lung cancer

Higher survival associated with low mRNA level							
No	**Gene symbol**	**Half-life (months)**				**P value**	
		AC		**SCC**		**AC**	**SCC**
		High (No.)	**low**	**high**	**low**		
1.	VDAC1	75 (360)	150 (360)	40 (262)	50 (262)	0.0018	0.87
2.	SMAC	65 (360)	120 (360)	60 (262)	60 (262)	7.4×10^{-6}	0.9
3.	HYOU1	65 (360)	115 (360)	60 (262)	60 (262)	2.2×10^{-6}	0.76
4.	TTLL12	75 (337)	120 (336)	50 (135)	55 (136)	2.9×10^{-4}	0.63
5.	RAB34	90 (337)	122 (336)	65 (135)	45 (136)	0.013	0.087
6.	MAVS	90 (337)	115 (336)	65 (135)	50 (136)	0.13	0.25
Higher survival associated with high mRNA level							
7.	ARL1	175 (360)	60 (360)	50 (261)	60 (263)	3.4×10^{-14}	0.84
8.	TSG101	137.5 (360)	65 (360)	50 (262)	50 (262)	1.9×10^{-9}	0.18
9.	HAT1	118 (360)	70 (360)	51 (262)	60 (262)	0.0046	0.32
10.	p40	115 (360)	70 (360)	60 (262)	60 (262)	1.4×10^{-7}	0.84
11.	NAPSA	130 (337)	80 (336)	65 (135)	50 (136)	4.2×10^{-5}	0.2
12.	LRRFIP2	120 (361)	75 (359)	52 (261)	52 (263)	0.02	0.51
13.	AIFM1	130 (360)	90 (360)	60 (262)	55 (262)	0.033	0.9
14.	TITF1	127 (360)	81.3 (360)	50 (262)	50 (262)	0.00051	0.11
15.	WDR82	120 (360)	90 (360)	50 (262)	50 (262)	2×10^{-4}	0.95

Data were obtained from KMplot.com. Total sample number was 2437, with initial number in each group presented in parenthesis. The Kaplan–Meier estimator used an earlier (2015) release of the database [130].

Supplementary Table 9: Selected biomarkers for distinguishing between AC and SCC

Protein	SCC/AC		Marker for:
Method:	**Proteomics (q-PCR)**	**RNA Seq**	
1. HAT1 - Histone acetyltransferase type B	475 (1.8)	1.4	SCC, this study
2. AKR1B10 - Aldo-keto reductase family 1 member B10 (*secreted*)	17.9 (20)	4	SCC, this study
3. USP14 - Ubiquitin carboxyl-terminal hydrolase 14	4.4 (2)	1.3	SCC, this study
4. TTLL12 - Tubulin-tyrosine ligase-like protein 12	8.1 (5)	2.5	SCC, this study
5. LRRF2 - Leucine-rich repeat flightless-interacting protein 2	19.3 (3)	1.1	SCC, this study
6. WDR82 - WD repeat-containing protein 82	9.8 (4.4)	1	SCC, this study
7. IGF2BP3 - Insulin-like growth factor 2 mRNA-binding protein	4.9	1.8	SCC, this study
8. ITGA7 - Integrin alpha-7 (Q13683)	107	-1.4	SCC, this study
9. PFN2 - Profilin-2	4.9	2.7	SCC, this study
10. Ck5 - Keratin, type II cytoskeletal 5	4.6	67.5	SCC [19, 120, 131]
11. Ck13 - Keratin, type I cytoskeletal 13	4.4	63.7	SCC [121]
12. Ck14 - Keratin, type I cytoskeletal 14	3.2	59.2	SCC [120, 122]
13. Ck17- Keratin, type I cytoskeletal 17	2.1	19.5	SCC [120]
14. GGH Gamma-glutamyl hydrolase *(secreted)*		1.9	SCC
15. SMAC - Second mitochondria-derived activator of caspases	**Nuclear localization in SCC**		SCC, this study
16. TSG101 - Tumor susceptibility gene 101 protein	-38.3 (-1.6)	-1.1	AC, this study
17. NAPSA - Napsin A aspartic peptidase	-	**-10**	AC [131]
18. ACOT1 - Acyl-coenzyme A thioesterase 1	-2.8	-1.7	AC, this study
19. RAB34 - Ras-related protein Rab-34	-6.0	-1.3	AC, this study
20. FEN1 - Flap endonuclease 1	3.5	1.5	AC [118]
21. AZGP1 - Zinc-alpha-2-glycoprotein *(secreted)*	-2.7	-4.1	AC [115]
22. ACAD8 - Isobutyryl-CoA dehydrogenase, mitochondrial	-17	-2.4	AC, this study
23. NPC2 Epididymal secretory protein E1 *(secreted)*		-2.1	AC [116]

Proteins that can be used as biomarkers are presented, with their expression levels in SCC, relative to AD, as determined by proteomics, qPCR (in parenthesis) and RNASeq studies, listed. The source of the data is also indicated. The proteins secreted to the blood are indicated, AKR1B10 [132], NPC2, [133], GGH [134] and AZGP1 [135].