Supplementary Materials

Metabolomic Analysis of Actinic Keratosis and SCC Suggests a Grade-Independent Model of Squamous Cancerization

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More than 40 metabolites in the tissues were identified; all the assignments are listed in Table S1. In the table are reported all the signals coming from mepivacaine, a local anesthetic used during surgery.

Table S1. List of ^{1}H and ^{13}C chemical Shift (δ, ppm) of metabolites. [a],^{1}H chemical shifts refer to Ala doublet at 1.48 ppm; [b],^{13}C chemical shifts refer to Ala at 19.3 ppm; [c], contributes to the 3.77, 57.1 ppm cross-peak.

	Metabolites	$\delta^{1}H$[a]	$\delta^{13}C$[b]	Assignment
1	Fatty acids	0.89	16.5	CH_3
		1.32–1.29	25.4, 32.6–34.2	$(CH_2)_n$
		1.59	27.5	CH_2-CH_2-C=O
		2.04	29.6	CH_2CH=CH
		2.27	36.1	CH_2-C=O
		2.78	27.9	=CH-CH_2-CH=
		5.32	128–130	-CH=CH-
2	Lactate	1.33 (d)	22.7	CH_3
		4.12 (q)	71.3	CH
3	Alanine	1.48 (d)	19.3	CH_3
		3.78	c	α CH
4	Valine	0.98 (d)	21.7	γ–CH_3
		1.04 (d)	20.7	γ–CH_3
		2.27	30.5	β–CH

		3.61	c	α-CH
5	Leucine	0.96	24.9	δ-CH_3
		0.97	25.3	δ–CH_3
		1.70	42.4	β–CH_2
		1.73	28.8	γ–CH_2
		3.72	c	α–CH
6	Lysine	1.47	22.6	γ–CH_2
		1.73	27.3	δ–CH_2
		1.88	32.9	β–CH_2
		3.03	39,7	ε–CH_2
		3.73	c	α–CH
7	Isoleucine	1.00 (d)	15.5	γ–CH_3
		0.95–0.93 (t)	11.7	δ–CH_3
8	Acetate	1.92 (s)	26.6	CH_3
9	Arginine	1.72,1.67		γ–CH_2
		1.91		β–CH_2
		3.25		δ–CH_2
		3.76	c	α-CH
10	N-acetyl	2.06 (s)	24.8	CH_3
11	Glutamine	2.13	29.0	β–CH_2
		2.45	33.4	γ–CH_2 (td)
		3.76	c	α–CH
12	Methionine	2.13 (s)	17.1	SCH_3
		2.62 (t)		γ-CH_2
		2.16		β-CH_2

		3.87		α-CH
13	Glutamate	2.36	36.4	γ–CH_2
		2.13,2.07	29.2	β–CH_2
		3.73	c	α–CH
14	Proline	3.43, 3.34		δ-CH_2
		2.01		γ-CH_2
		2.34, 2.08		β-CH_2
		4.12		α-CH
15	Threonine	1.32	21.8	γ–CH_3
		3.56	69	β–CH
		4.25	66.9	α–CH
16	α-glucose	5.24 (d)	92.6	1 CH
		3.55	72	2 CH
		3.72	73.1	3 CH
		3.42	76.4	4 CH
		3.82	71.9	6 CH_2
17	β-glucose	4.64 (d)	96.4	1 CH
		3.25	74.7	2 CH
		3.49	76.3	3 CH
		3.4	70.2	4 CH
		3.47	76.4	5 CH
		3.91	61.3	6 CH_2
18	Ribose	5.80		
19	Creatine	3.03 (s)	39.6	CH_3
		3.95 (s)	56.4	CH_2
20	Creatinine	3.05 (s)		CH_3

		4.06 (s)		CH_2
21	Ethanolamine	3.15 (t)		CH_2
		3.82 (t)		CH_2
22	Histidine	3.19		β-CH_2
		3.97		α-CH
		7.05 (s)		2-CH
		7.78 (s)		4-CH
23	Phenylalanine	3.28, 3.11		β-CH_2
		3.99		α-CH
		7.34	130.1	H-*ortho*
		7.37		H-*para*
		7.43	129.6	H-*meta*
24	Choline	3.20	56.6	$N(CH_3)_3$
		3.50	68.2	NCH_2
		4.08	56.5	OCH_2
25	Glycine	3.56 (s)	44.3	CH_2
26	Taurine	3.42 (t)	38.2	N-CH_2
		3.26 (t)	50.2	S-CH_2
27	Tyrosine	6.88		5 CH
		7.18		6 CH
28	Succinate	2.37 (s)	34.2	CH_2
29	Glycerol bound	4.30, 4.10	64.7	CH_2
		5.22	71.7	CH
30	Free Glycerol	3.56-3.65	63.3	1-CH_2
		3.81	72.7	2-CH
31	Glyceryl phosphorylcholine	4.32	60	α'-CH_2
		3.67	66.6	γ–CH_2
		3.24 (s)	56.5	N-CH_3

32	Phosphorylcholine	3.22	54.7	$N(CH_3)_3$
		3.61	67.3	NCH_2
	Aspartate	4.22	59.0	OCH_2
33		2.68–2.82	37.2	β-CH_2
		3.90		α-CH
34	Asparagine	2.85-2.96	26.3	β-CH_2
		4.01		α-CH
35	Pyruvate	2.39 (s)	29.1	CH_3
36	Pyroglutamic acid	2.03, 2.51	27.9	2,4 CH_2
		2.41	32.5	3 CH_2
		4.19	61.2	1 CH
37	Myo-inositol	3.53	72	1,3 CH
		4.06	71.3	2 CH
		3.63	73.1	4,6 CH
		3.27	74.4	5 CH
38	Scyllo-inositol	3.36 (s)	73.6	CH
39	Serine	3.87	59.2	α–CH
		3.94	63.2	β–CH_2
40	EtOH	1.18	17.5	CH_3
		3.65	56.7	CH_2
41	Glutathione	2.55		5 CH_2
		2.95		4 CH_2
		4.57		1 CH
42	Ascorbate	4.52		4 CH

		4.01	5-CH
43	Formate	8.48	$HCOO^-$
44	Mepivacaine	2.18 (bs)	CH_3
		2.23 (bs)	CH_3
		7.21 (d)	3,5-CH
		7.25 (t)	4-CH
45	Fumarate		

Table S2. *p values* derived from the comparison between healthy and AKI, healthy and AKII, healthy and AKIII and finally healthy and SCC.

Metabolites	**Healthy vs :**			
	P value AKI	**P value AKII**	**P value AKIII**	**P value SCC**
Glc	0,3245	0,6092	0,0662	0,1344
GSH	0,0359	0,1543	0,0542	0,1664
Asc	0,0021	0,0000	0,0034	0,0913
Lac	0,0002	0,0005	0,0007	0,0077
Ser	0,0652	0,2487	0,0220	0,0094
Gly	0,0715	0,0739	0,0011	0,0416
Myo	0,2300	0,5725	0,0746	0,2561
Tau1	0,0075	0,0036	0,0245	0,0127
Scy	0,1675	0,1946	0,1142	0,2681
Tau2	0,0111	0,0020	0,0962	0,0348
GPC	0,0882	0,1198	0,0110	0,0221
PC	0,0181	0,0094	0,0111	0,0886
Cho	0,3953	0,6824	0,2268	0,0281
EtA	0,0205	0,0574	0,0015	0,0019
Cr	0,3076	0,1712	0,0016	0,1501
Gln	0,0013	0,0006	0,0001	0,0012
PGA	0,2173	0,9849	0,0204	0,0171
Glu	0,0026	0,0004	0,0010	0,0015
Met	0,0010	0,0159	0,0158	0,0153
Nacetyl		0,6424	0,9933	0,4908
Ac	0,6520	0,6412	0,0378	0,2958
Ala	0,1195	0,0956	0,0232	0,0206
Val	0,0315	0,0530	0,0022	0,0305

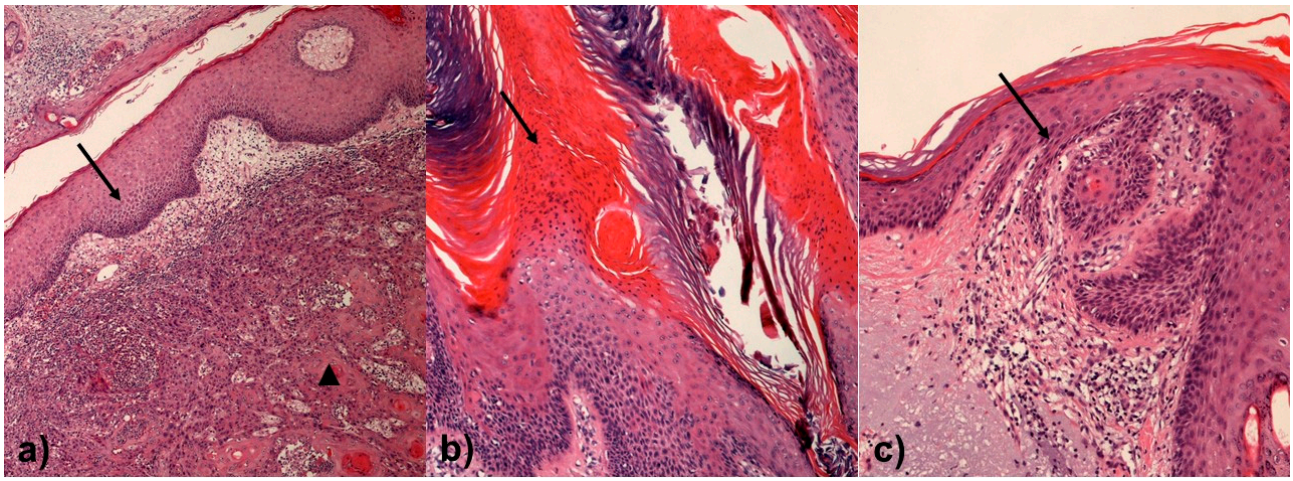

Figure S1. Selected histopathological section (hematoxylin and eosin, ×100 (A) and ×40 (B,C) magnification) corresponding to: (A) moderately differentiated SCC with Breslow thickness of 5 mm (triangle), associated with AK I (arrow); (B) AK III showing parakeratosis (arrow); (C) AK I characterized by the presence of hypertrophy (arrow).