cancers

MDPI

Article

Mass Spectrometry Imaging for Reliable and Fast Classification of Non-small Cell Lung Cancer subtypes

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Supplementary Materials

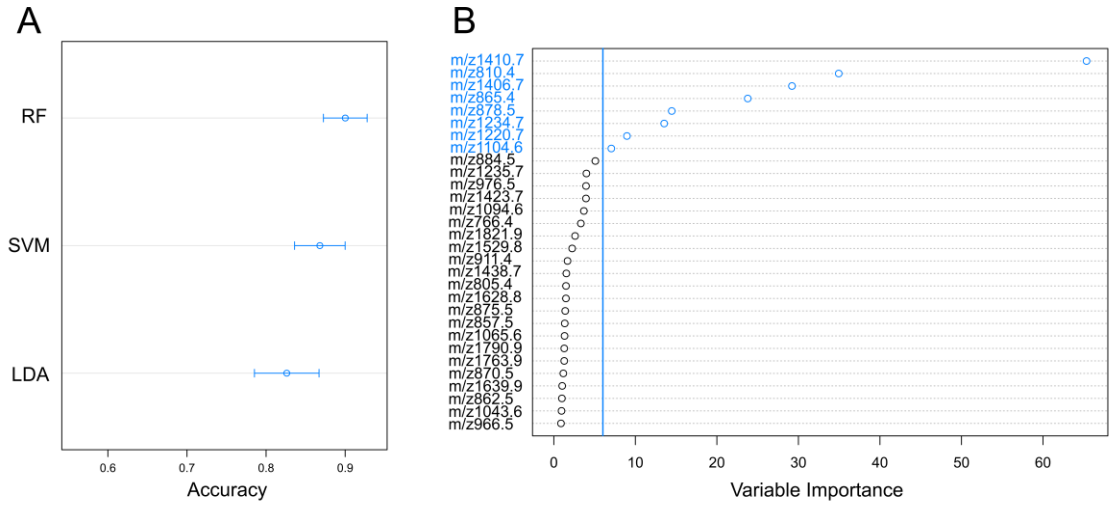
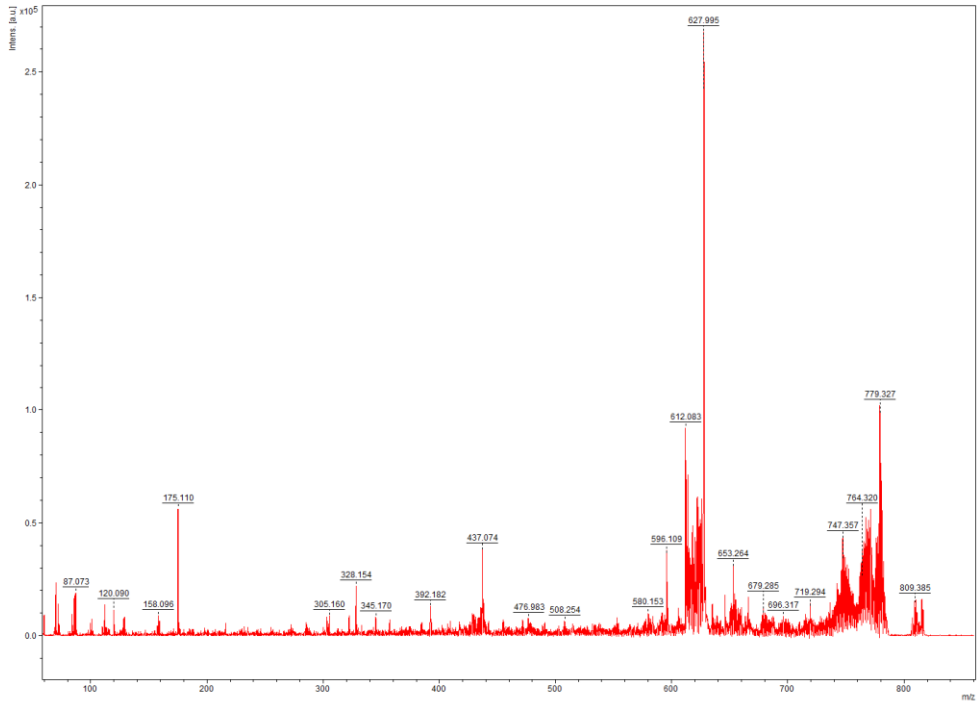
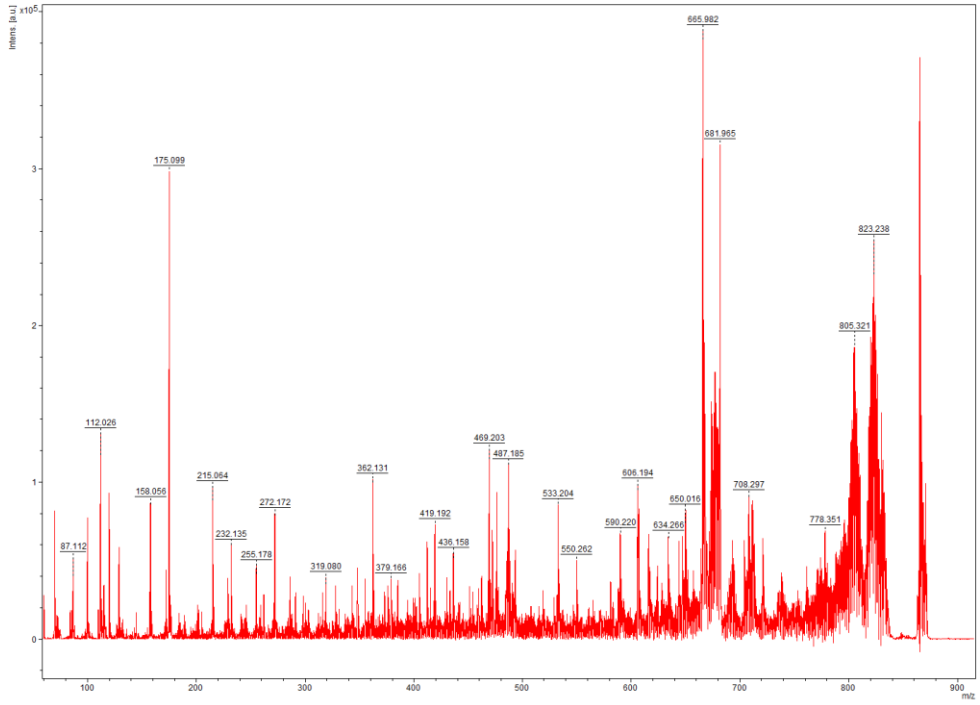


Figure S1. Classification accuracy and variable importance of different algorithms. Among the evaluated classification algorithms—RF (random forest), SVM (support vector machine) and LDA (linear discriminant analysis)—RF showed the highest level of accuracy in discriminating between ADC and SqCC samples (**A**). *m/z* peaks were ranked by variable importance to contribute to the RF classification algorithm. The 8 most important *m/z* peaks were selected for further evaluation (**B**).


Intens. [a.u.]
x10^5
627.995
612.083
779.327
175.110
764.320
747.357
437.074
596.109
653.264
328.154
679.285
719.294
809.385
87.073
120.090
158.096
305.160
345.170
392.182
476.983
508.254
580.153
696.317
m/z


Intens. [a.u.]
x10^5
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681.965
175.099
823.238
805.321
112.026
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487.185
362.131
215.064
606.194
158.056
533.204
650.016
708.297
272.172
419.192
590.220
634.266
778.351
232.135
436.158
87.112
255.178
550.262
319.080
379.166
m/z

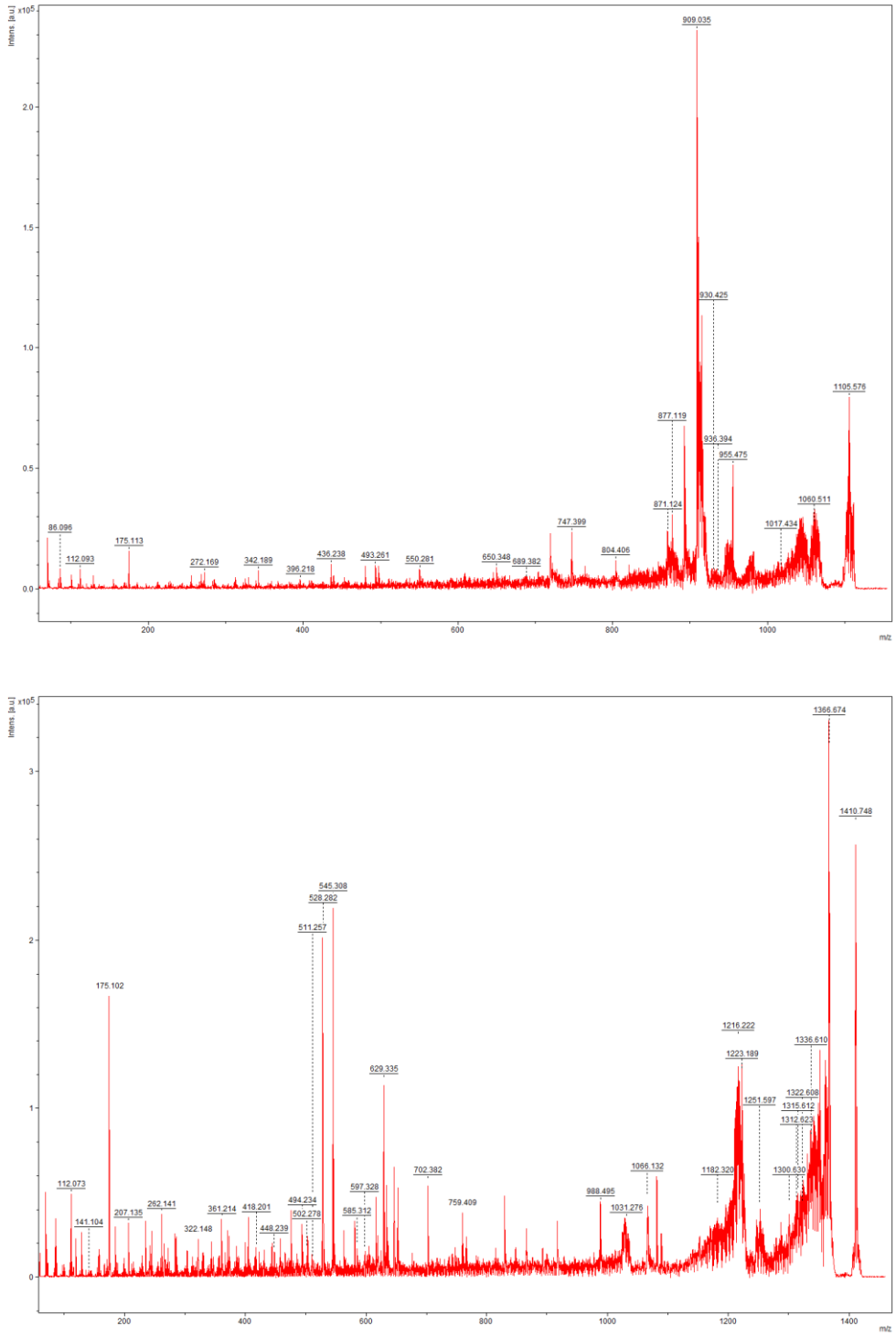

Figure S2. Spectra from Identifications.

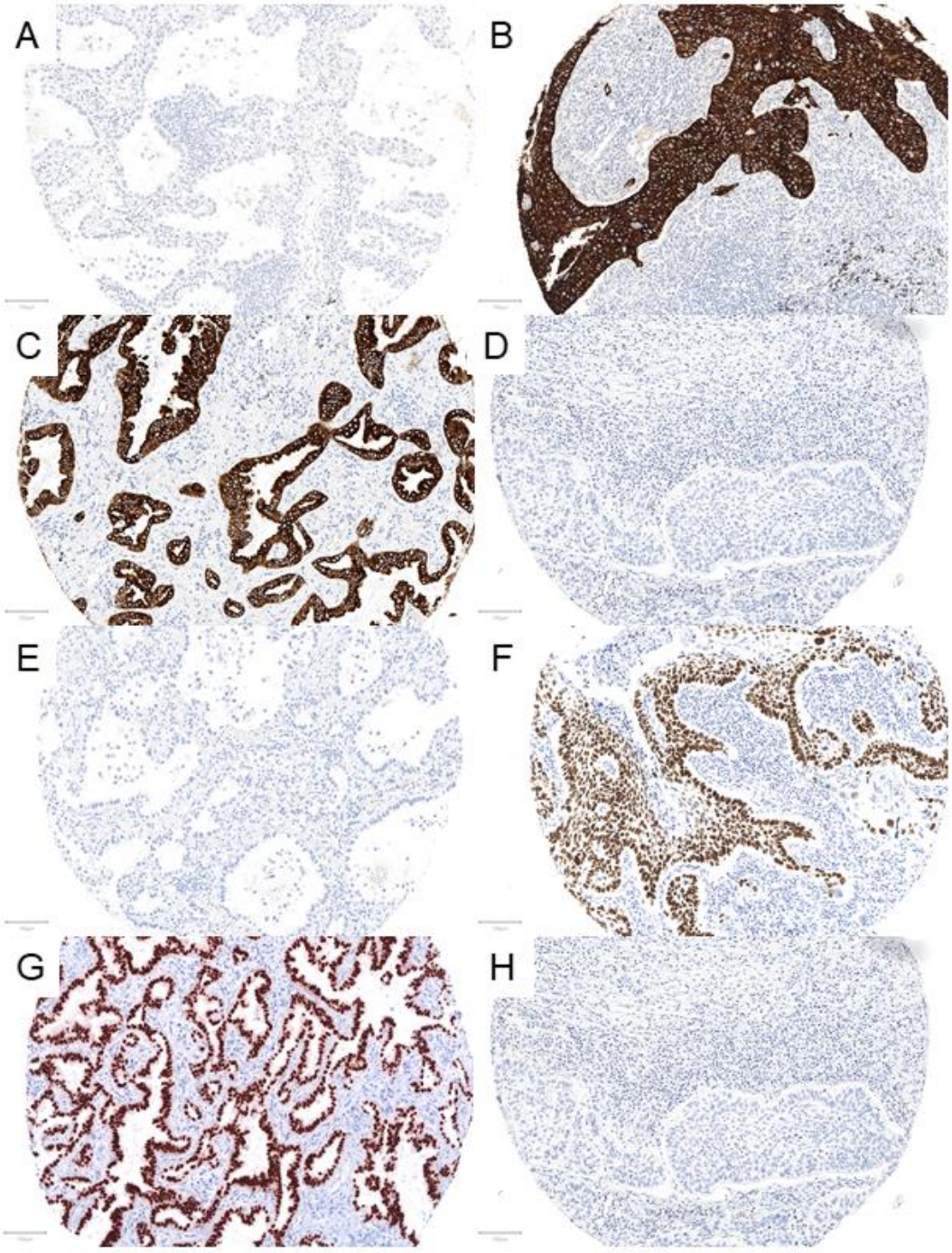

Figure S3. Typical examples of the immunohistological staining characteristics of adenocarcinoma and squamous cell carcinoma. Immunohistological staining of adenocarcinoma (left column) and squamous cell carcinoma (right column) are displayed. Adenocarcinomas are frequently positive for CK7 (**C**) and TTF-1 (**G**) and are negative for CK5/6 (**A**) and p40 (**E**). Squamous cell carcinoma is often positive for CK5/6 (**B**) and p40 (**F**) and is frequently negative for CK7 (**D**) and TTF-1 (**H**).

Table S1. MS/MS identification results.

Observed *m/z*	Mr (expt) *m/z*	Mr (calc) *m/z*	Mass Error (ppm)	Mascot Score	Peptide Sequence	Protein Name	Variable Modifications
Identified in this study							
810.4	809.3	809.4	−6.7	64	R.QSSVSFR.S	Keratin 5, type II	Acetyl (Protein N-term), Oxidation (M)
865.3	864.3	864.3	9.1	47	R.SGGGGGGGFGR.V	Keratin 5, type II	Acetyl (Protein N-term), Oxidation (M)
1104.5	1103.5	1103.5	−10.5	69	R.SAYGGPVGAGIR.E	Keratin 7, type II	Acetyl (Protein N-term), Oxidation (HW), Oxidation (M), Oxidation (P)
1410.7	1409.7	1409.7	26.7	42	R.SFSTASAITPSVSR.T	Keratin 5, type II	Acetyl (Protein N-term), Oxidation (M)
Identified in the literature							
1220.6 1222.6 [10]	1221.6	1221.6	−2.95	55	R.TKFETEQALR.M	Keratin 19, type I	
1222.6 [10]	1221.6	1221.6	−20.45	40	R.LEQEIATYR.R	Keratin 17, type I	
1234.6 1235.6#			−11.37	36	TGHPGTVGPAGIR	Collagen alpha-2(I) chain	
1406.7 1407.7 [11]	1406.7	1406.7	−1.01	34	K.ADTLTDEINFLR.A	Keratin 6A, type II	
1406.7 [11]	1405.7	1405.7	2.63	61	M.SIHFSSPVFTSR.S + Acetyl (Protein N-term)	Keratin 7, type II	
Not identified							
878.4							

Table S2. Antibodies and staining conditions.

Antibody	Company	Clone	Pretreatment	Buffer Incubation Time (min)	Antibody Incubation Time (min)	Dilution
CK5/6	Ventana	D5/16 B4	Tris/Borat/EDTA, pH 8.4	56	24	RTU
p40	Ventana	BC28	Tris/Borat/EDTA, pH 8.4	48	24	RTU
CK7	Ventana	SP52	Tris/Borat/EDTA, pH 8.4	32	24	RTU
Napsin-A	Novocastra	IP64	Tris/Borat/EDTA, pH 8.4	32	24	1:400
TTF-1	Novocastra	SPT24	Tris/Borat/EDTA, pH 8.4	56	24	1:100

CK, cytokeratin; RTU, ready to use; TTF-1, thyroid transcription factor-1.