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Article

Quantitative proteomics identifies biomarkers to distinguish pulmonary from head and neck squamous cell carcinomas by immunohistochemistry.

Richter A, Fichtner A, Joost J, et al. J Pathol Clin Res. 2022 Jan;8(1):33-47. From Germany

頭頸部扁平上皮癌（HNSCC）の既往のある患者の肺に生じた扁平上皮癌は、それが肺原発（SQCLC）か転移か鑑別することが難しい。しかしながら予後と治療方針に大きく関わってくる。筆者らはhigh-resolution mass spectrometryを用いて、HNSCC細胞株とSQCLC細胞株で発現が異なっている379のタンパク質を同定した。それらの中で鑑別に有用と考えられた6つのタンパク質（CAV1, CAV2, LGALS1, LGALS7, CK19 and UGDH）の発現を免疫組織化学で検討した論文である。

免疫組織化学の評価

Immunoreactivity staining score (IRS)

陽性細胞率を0-4に分ける(0:0%, 1:10%未満、2：10-50%, 3:51-80%, 4:80%超)

染色強度を0-3に分ける

両者を乗算し　0-1:Negative, 2-3:weakly positive, 4-6:moderately positive, 8-12:strongly positiveとし

IRS≥2を陽性として検討。

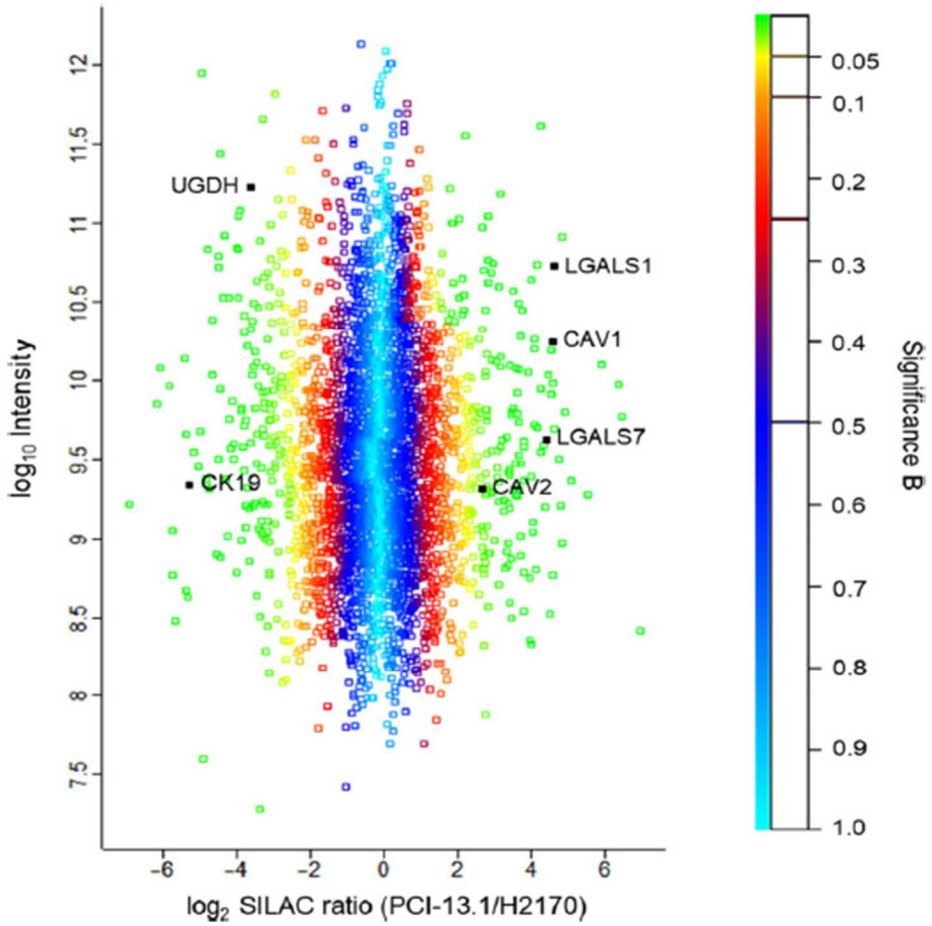


Figure 1. Analysis of the significance of the SILAC-based

HNSCC細胞株(PCI-13.1)とSQCLC細胞株(H2170)を用いて発現しているproteinを比で検討。緑点が両者で明らかに異なった発現量を示したprotein(n=379)。この中からHuman Protein Atrasの発現データと比較して6つの候補マーカーを選んだ。

Table 1. Proteins selected for immunohistochemistry with their log_2 SILAC ratios (PCI-13.1/H2170), log_{10}-transformed intensity values, and calculated *P* values. The original data are in supplementary material, Table S3.

Protein	Gene	Log_2 SILAC ratio (PCI-13.1/H2170)	Log_{10}- intensity	*P* value
Caveolin-1	*CAV1*	2.30	10.247	4.50E-05
Caveolin-2	*CAV2*	1.32	9.311	3.19E-02
Galectin-1	*LGALS1*	2.32	10.726	4.14E-11
Galectin-7	*LGALS7*	2.20	9.624	6.00E-04
Cytokeratin-19	*KRT19*	−2.64	9.33638	2.12E-05
UDP-glucose 6-dehydrogenase	*UGDH*	−1.80	11.2308	6.63E-03

これらのうち上記4つはHNSCCでより発現しているもの、残りの2つはSQCLCでより発現しているものである。

Table 2. Results of the marker candidates in the HPA and cohort 1.

Marker	SILAC-MS (significantly stronger in)	IHC (HPA) (*n*) positive		IHC (cohort 1) (*n*) positive	
CAV1	PCI-13.1 (HNSCC)	3/3	HNSCC	6/6	HNSCC
		1/5	SQCLC	0/6	SQCLC
CAV2	PCI-13.1 (HNSCC)	3/3	HNSCC	6/6	HNSCC
		1/4	SQCLC	1/6	SQCLC
LGALS1	PCI-13.1 (HNSCC)	3/4	HNSCC	5/6	HNSCC
		1/5	SQCLC	1/6	SQCLC
LGALS7	PCI-13.1 (HNSCC)	3/3	HNSCC	6/6	HNSCC
		1/4	SQCLC	1/6	SQCLC
CK19	H2170 (SQCLC)	1/4	HNSCC	2/6	HNSCC
		3/4	SQCLC	6/6	SQCLC
UGDH	H2170 (SQCLC)	1/3	HNSCC	2/6	HNSCC
		5/6	SQCLC	5/6	SQCLC

The table shows the results of the SILAC-MS as well as those of the IHC in the HPA and cohort 1. The absolute number of positive cases and the total number of cases are given for each case. The proteins that were upregulated in HNSCC are highlighted in blue and the proteins that were downregulated are highlighted in grey.
HPA, Human Protein Atlas; IHC, immunohistochemistry; SILAC-MS, SILAC-based mass spectrometry.

少数サンプルでのテスト検討(HNSCC=6, SQCLC=6)。CAV1,2 LGALS1,7ではHNSCCで陽性率が高く、CK19, UGDHはSQCLCで陽性率が高かった。

Table 3. Clinical and pathological data.

		SQCLC ($n = 98$)	HNSCC ($n = 96$)
Age	Mean ± SD	65.5 ± 8.4	62.1 ± 10.4
	Age range	43–81	24–83
Sex	Male	86 (87.8%)	71 (74.0%)
	Female	12 (12.2%)	25 (26.0%)
Localisation	Oral cavity	–	48 (50.0%)
	Pharynx	–	25 (26.0%)
	Larynx	–	23 (24.0%)
	Lung	98 (100%)	–
pT stage	pT1	19 (19.4%)	22 (22.9%)
	pT2	63 (64.3%)	41 (42.7%)
	pT3	12 (12.2%)	19 (19.8%)
	pT4	4 (4.1%)	14 (14.6%)
pN stage	pN0	59 (60.2%)	52 (54.2%)
	pN1	22 (22.4%)	23 (24.0%)
	pN2	17 (17.3%)	21 (21.9%)
pM stage	pM0	98 (100%)	95 (99.0%)
	Uncertain pM1	0 (0%)	1 (1.0%)
UICC stage (eighth edition)	I	33 (33.7%)	16 (16.7%)
	II	43 (43.9%)	26 (27.1%)
	III	22 (22.4%)	25 (26.0%)
	IV	0 (0.0%)	29 (30.2%)
Grade	G1	0 (0.0%)	0 (0%)
	G2	70 (70.7%)	87 (90.6%)
	G3	28 (29.3%)	9 (9.4%)
p16 positive		–	12 (12.5%)

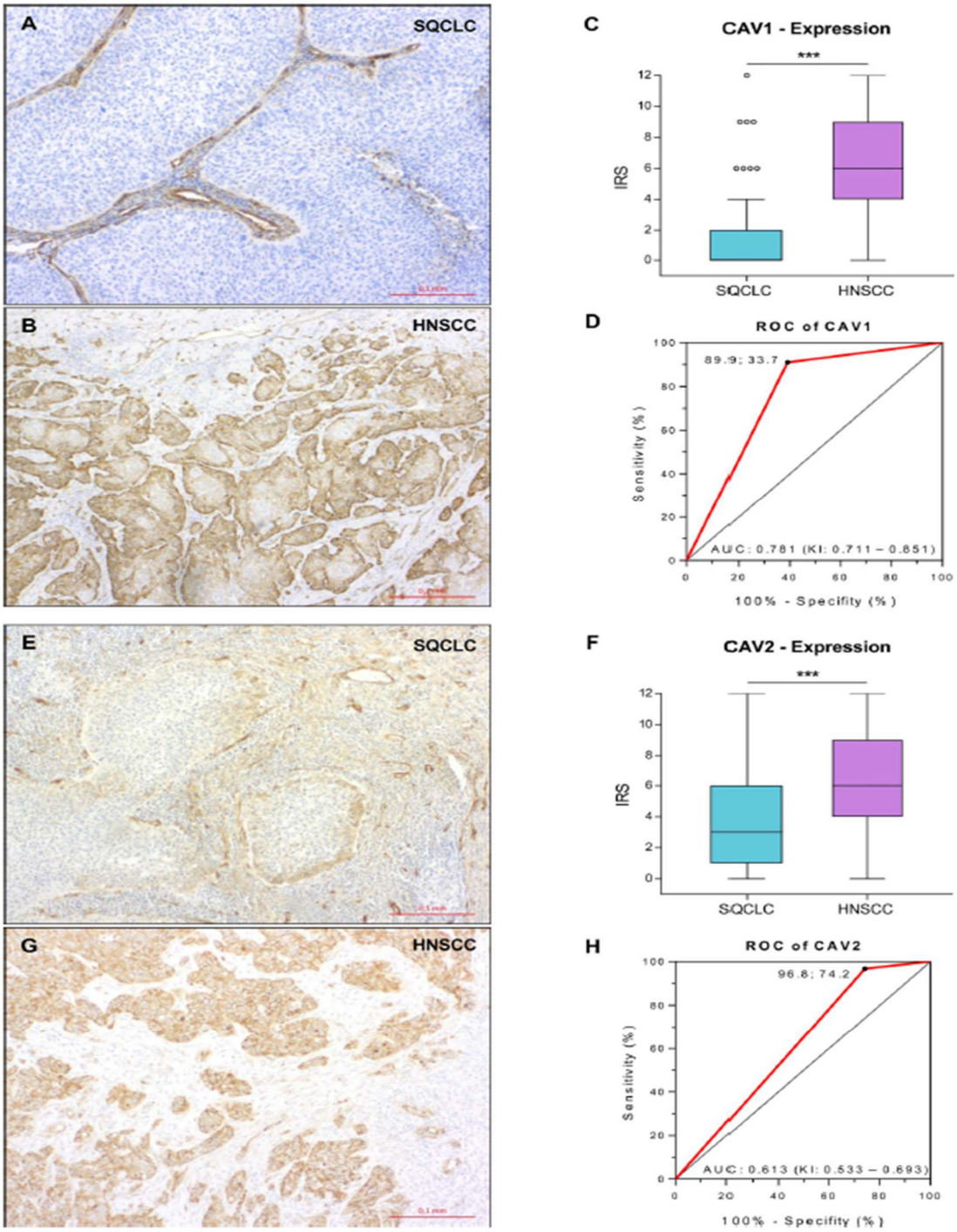
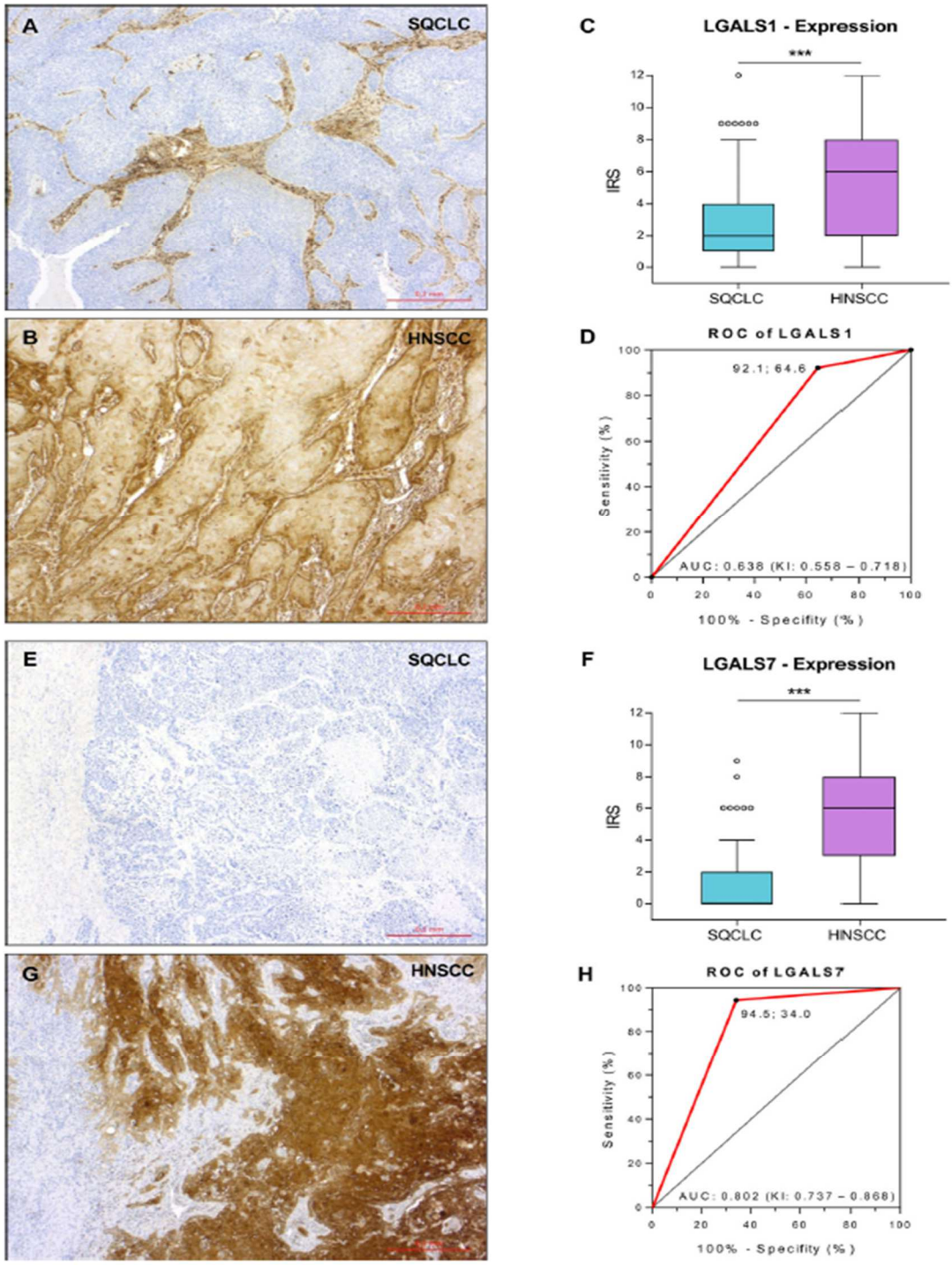


Figure 2. Legend on next page.

CAV1: HNSCC(89.9%) vs SQCLC(33.7%), Median IRS 6vs.0 AUC 0.781 感度:89.9%, 特異度:66.3%, 陽性的中率:72.1%,陰性的中率:87.1%

CAV2: HNSCC(96.8%) vs SQCLC(74.2%), Median IRS 6vs.3 AUC 0.613 感度:96.8%, 特異度:25.8%, 陽性的中率:55.6%,陰性的中率:89.3%



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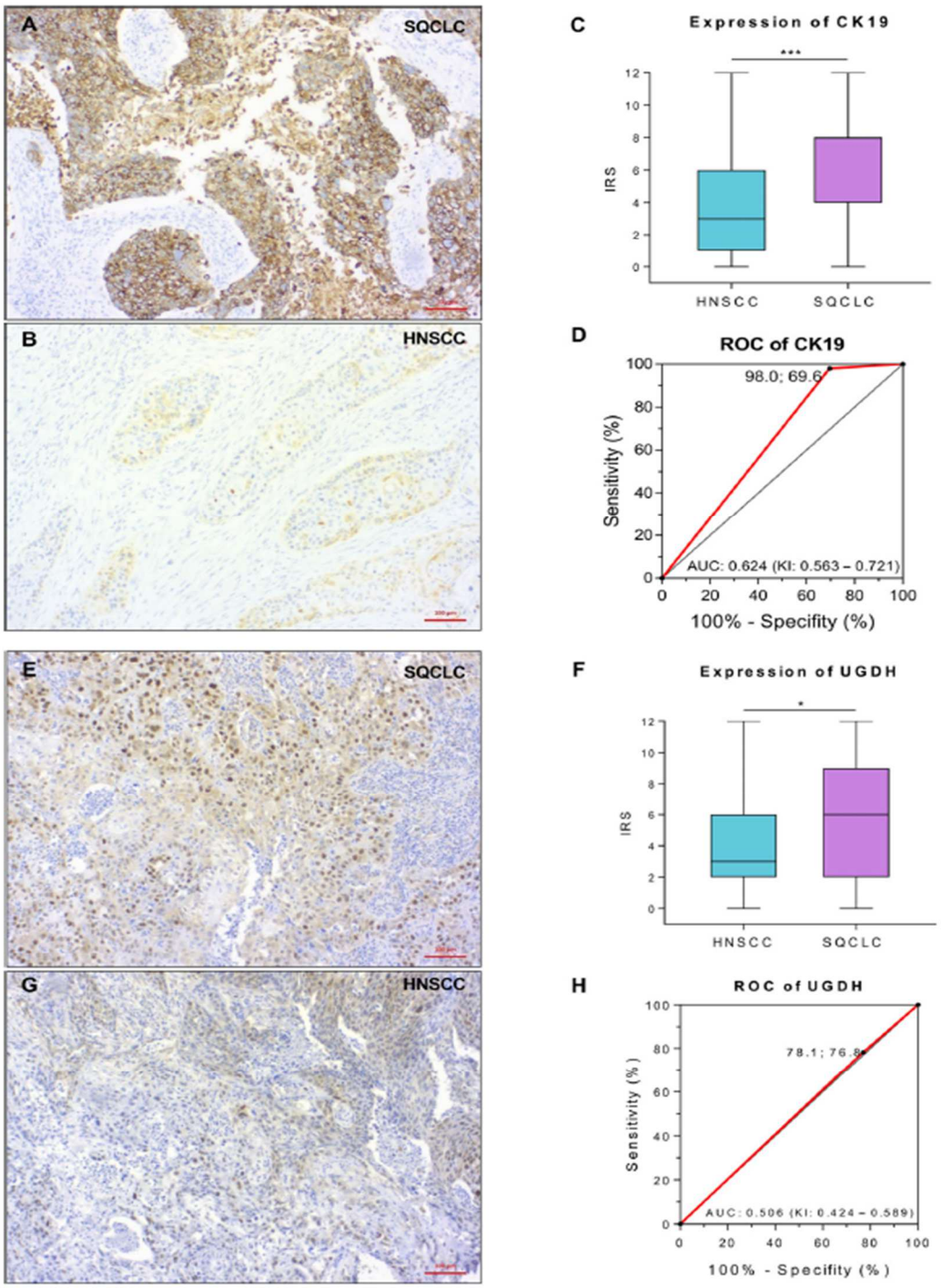
LGALS1: HNSCC(92.1%) vs SQCLC(64.6%), Median IRS 6vs.2 AUC 0.638 感度:92.1%, 特異度:35.4%, 陽性的中率:56.9%,陰性的中率:81.6%

LGALS7: HNSCC(94.5%) vs SQCLC(34%), Median IRS 6vs.0 AUC 0.802 感度:94.5%, 特異度:66%, 陽性的中率:72.3%,陰性的中率:92.3%



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CK19: HNSCC(69.5%) vs SQCLC(97.9%), Median IRS 3vs.8 AUC 0.624 感度:98%, 特異度:30.4%, 陽性的中率:60%,陰性的中率:93.3%

UGDH: HNSCC(76.8%) vs SQCLC(78.1%), Median IRS 3vs.6 AUC 0.506 感度:78.1%, 特異度:23.2%, 陽性的中率:50.7%,陰性的中率:51.2%

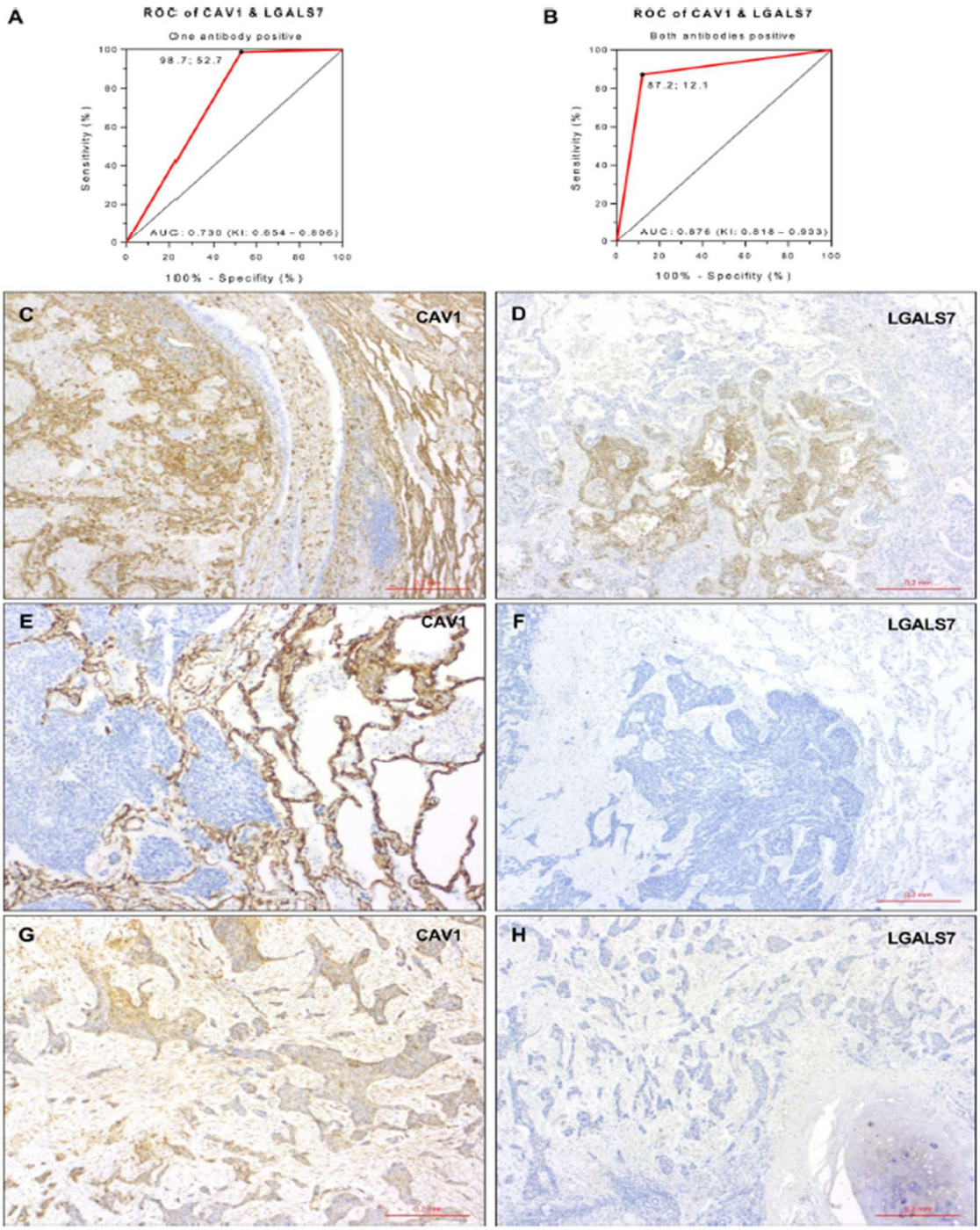

Figure 5. Legend on next page.

Either CAV1 or LGALS7: HNSCCの診断率はAUC 0.781 感度:98.7%, 特異度:47.3%, 陽性的中率:61%,陰性的中率:97.7%

Both CAV1 and LGALS7: HNSCCの診断率はAUC 0.876 感度:87.2%, 特異度:87.9%, 陽性的中率:86.1%,陰性的中率:88.9%

nistry of lung tumours of unknown origin in patients with prior HNSCC.

Localisation of HNSCC	Recurrence	Pulmonary foci (*n*)	Time interval (years)	pT	pN	Grade	CAV1	LGALS7	Clinical classification	IHC classification
Larynx	Yes	2	<1	4	2b	G2	*Positive*	*Positive*	met-HNSCC	*met-HNSCC*
Larynx	Yes	1	≥3	1	0	G2	*Positive*	Negative	met-HNSCC	Uncertain
Oral cavity	Yes	1	>3	3	1	G2	*Positive*	*Positive*	met-HNSCC	*met-HNSCC*
Oropharynx	No	2	<1	2	0	G2	Negative	Negative	met-HNSCC	SQCLC
Hypopharynx	No	1	<3	3	1	G2	*Positive*	Negative	SQCLC	Uncertain
Larynx	No	1	<1	2	2b	G2	Negative	Negative	met-HNSCC	SQCLC
Hypopharynx	No	1	<3	4	2b	G2	*Positive*	*Positive*	SQCLC	*met-HNSCC*
Larynx	No	1	<3	4	2c	G2	*Positive*	*Positive*	SQCLC	*met-HNSCC*
Larynx	No	1	<3	2	0	G2	*Positive*	*Positive*	SQCLC	*met-HNSCC*
Oral cavity	No	1	<1	2	2c	G2	*Positive*	Negative	met-HNSCC	Uncertain
Oropharynx	No	1	<1	2	1	G2	*Positive*	*Positive*	met-HNSCC	*met-HNSCC*
Oropharynx	No	1	<3	3	2c	G3	*Positive*	*Positive*	SQCLC	*met-HNSCC*

(Tu-01–Tu-12) of unknown origin, those of the previously known HNSCC (localisation, relapse, pT, pN, grade) as well as the results of the IHC staining of the lung tumours are pres- and the classification as SQCLC are marked in bold and the positive results and the classification as met-HNSCC in italics.
nohistochemical.

HNSCCの既往がある患者に出現した肺の扁平上皮癌（unknown origin）12症例に対し、CAV1とLGALS7の組み合わせによりIHCを行った。Double positiveとなったものをHNSCCの転移、double negativeとなったものをprimary SQCLC、それ以外をUncertainとした。

Take home message

・CAV1とLGALS7の発現状態を調べることにより、肺原発扁平上皮癌かHNSCCの肺転移かを鑑別することができる可能性が示唆された。

Caveolin-1 (CAV1) belongs to a group of caveolins and is an integral membrane protein. As part of the caveolin scaffolding domain, which are cytoskeletal associated proteins, it links cell adhesion molecules and signaling molecules. Therefore, it participates in multiple processes of malignant tumour cells including signal transduction, cell transformation, cell migration, and metastasis

Galectin-7 (LGALS7) has a high specificity for squamous epithelium and is physiologically expressed in all squamous epithelia and myoepithelium of the breast. It takes part in differentiation and development of epithelia as well as tissue repair, cell–cell interaction, cell–matrix interaction, and apoptosis. LGALS7 has positive and negative regulating functions depending on the tumour entity

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