

Pan-cancer tissue microarray analysis identifies Uroplakin 1b as a putative diagnostic marker in surgical pathology

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Introduction and Objectives

Uroplakin 1B (Upk1b), is a 29.6 kDa protein encoded by the UPK1B gene located at 3q13.3-q21. Upk1b is one out of 5 known uroplakin (Upk) protein particles that jointly form apical asymmetrical unit membrane (AUM) plaques which are thought to play an important role in stabilizing and strengthening epithelial cells that line the urinary bladder and thus enable the inner bladder membrane to stretch and prevent urothelial cells from rupturing during bladder distension. Studies and databases summarizing RNA expression of genes in different normal tissues have suggested that Upk1b expression occurs in a limited number of organs including urinary bladder, kidney, prostate, gallbladder, stomach, placenta, fallopian tube, uterine cervix, and tonsils. Only few studies addressed the role of Upk1b in cancer. It was the aim of this study to establish a catalogue of UPK1B expression in human normal and cancerous tissues.

Materials & Methods

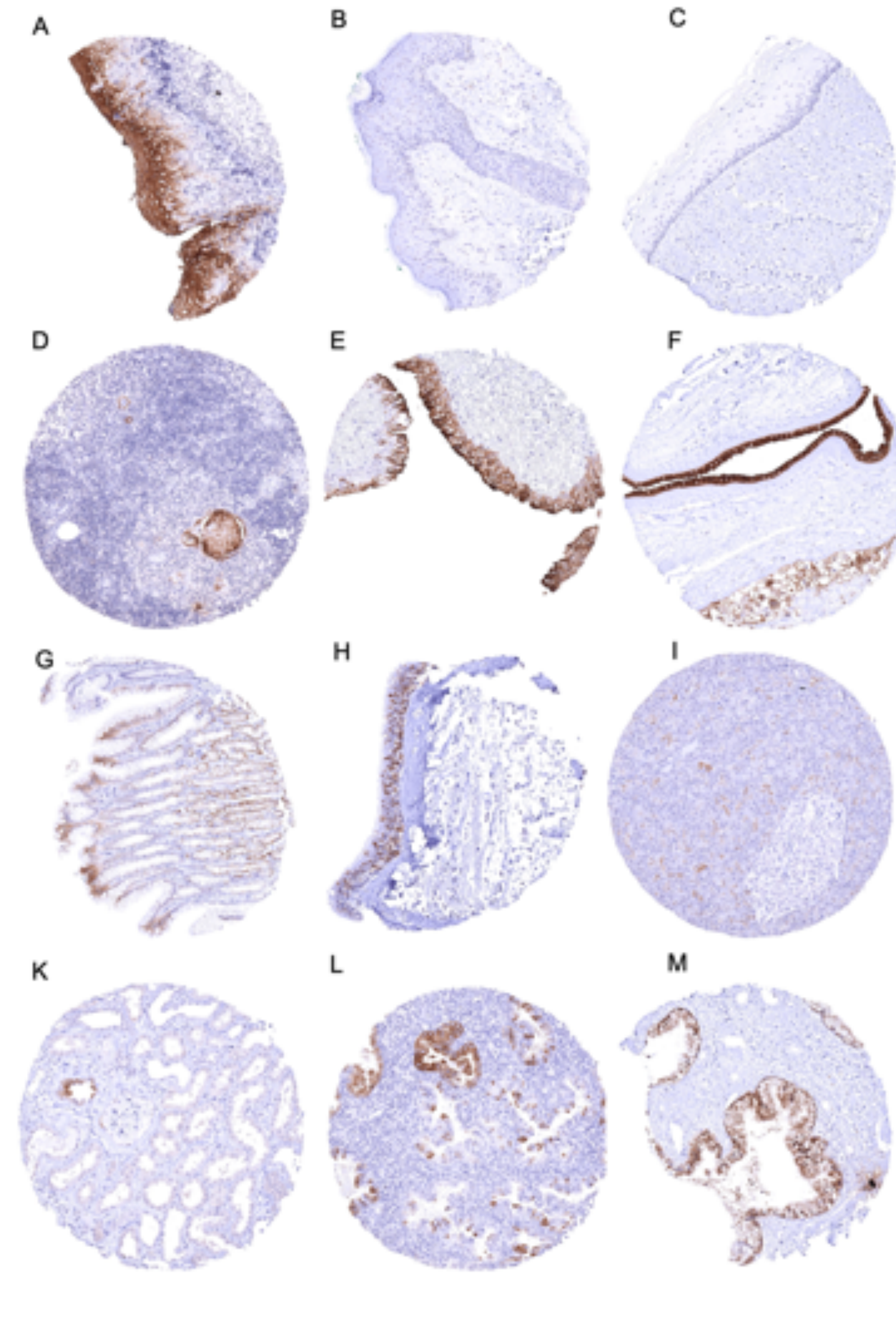
To comprehensively evaluate the potential diagnostic and prognostic utility of Upk1b expression analysis, a tissue microarray containing 15,182 samples from 127 different tumor types and subtypes and 608 samples of 76 different normal tissue types was analyzed by immunohistochemistry.

Immunostaining protocol and controls



- Antibody: clone MSVA-734M, MS validated antibodies, Mouse IgG, Dilution: 1:150
- Antigen retrieval: 5 min at 121°C (autoclave) in pH 7.8 buffer
- Controls:
 - Positive: Strong membranous and cytoplasmic UPK1b immunostaining in the urothelium, limited to the top cell layers or present in all cell layers.
 - Negative: UPK1b immunostaining should be absent in all cells of the colon mucosa.

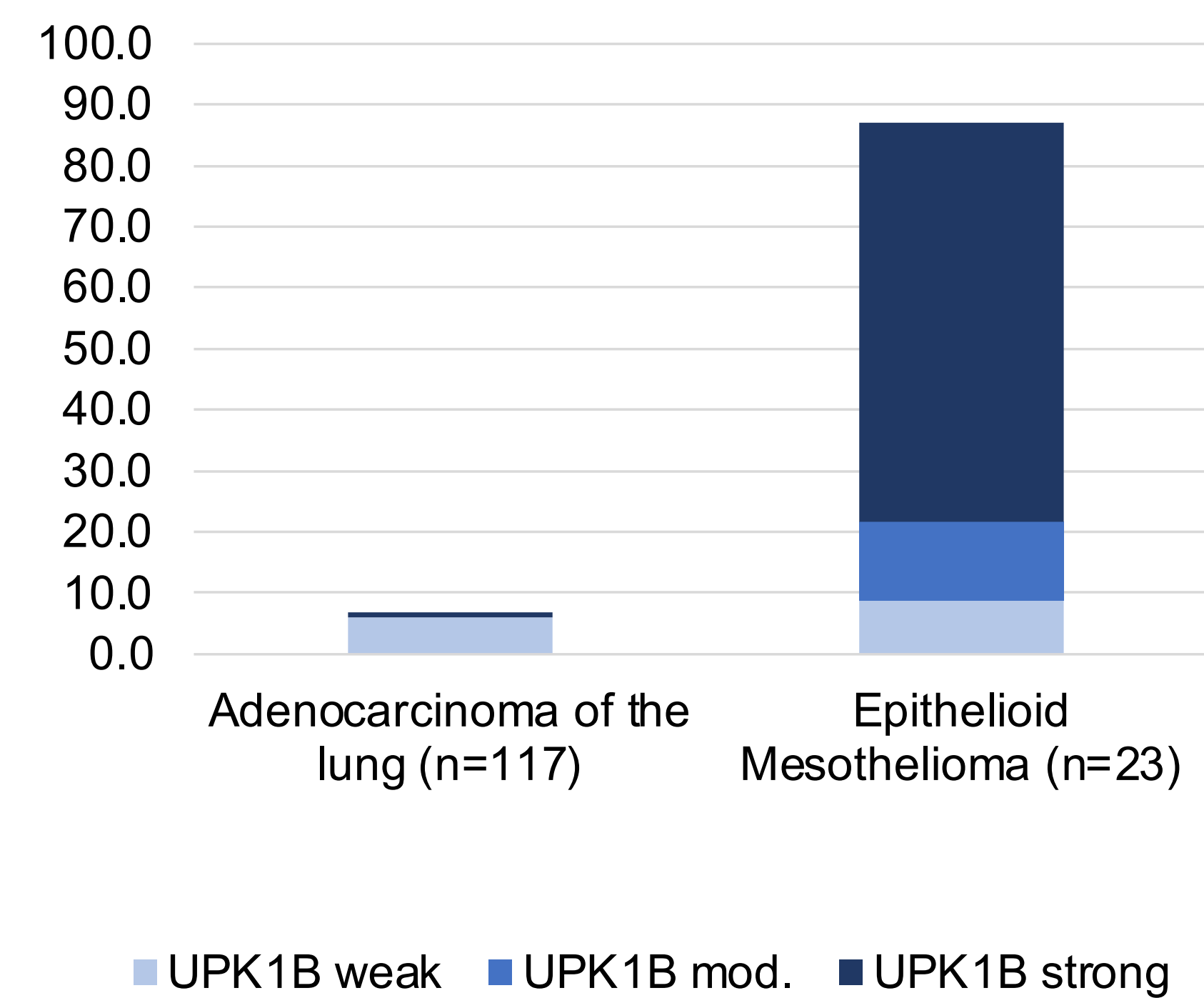
UPK1b in normal tissues



A strong membranous and cytoplasmic UPK1b immunostaining is seen in the urothelium (E). Upk1b was also regularly expressed in tonsil surface epithelium (A), tonsil crypt epithelium and in a fraction of cells in corpuscles of Hassall's in the thymus (D) as well as (faintly) other thymic epithelial cells. A moderate to strong Upk1b positivity also occurred in a fraction of cells of the respiratory epithelium (H), amnion and chorion cells of the placenta (F), gallbladder epithelium, intrahepatic bile ducts, some endocervical (M) and endometrial (L) glands, a fraction of cells in the fallopian tube, intercalated ducts (I) and a fraction of large excretory ducts of the pancreas, superficial epithelial cells (e.g. skin (B) and cervix (C)) and parietal cells of the stomach (G), the parietal layer of Bowman capsule in the kidney (K), and the superficial layer of anal transitional epithelium.

Useful for diagnosis of malignant mesothelioma

The striking difference in the Upk1b positivity rate between mesotheliomas containing an epithelioid tumor component (87%) and lung adenocarcinomas (7%) strongly suggests that addition of Upk1b might strengthen the diagnostic accuracy of currently applied panels of WT-1, Calretinin, D2-40, Ber-EP4, TTF-1, BAP-1 and Claudin-4 for the diagnosis of malignant mesothelioma.

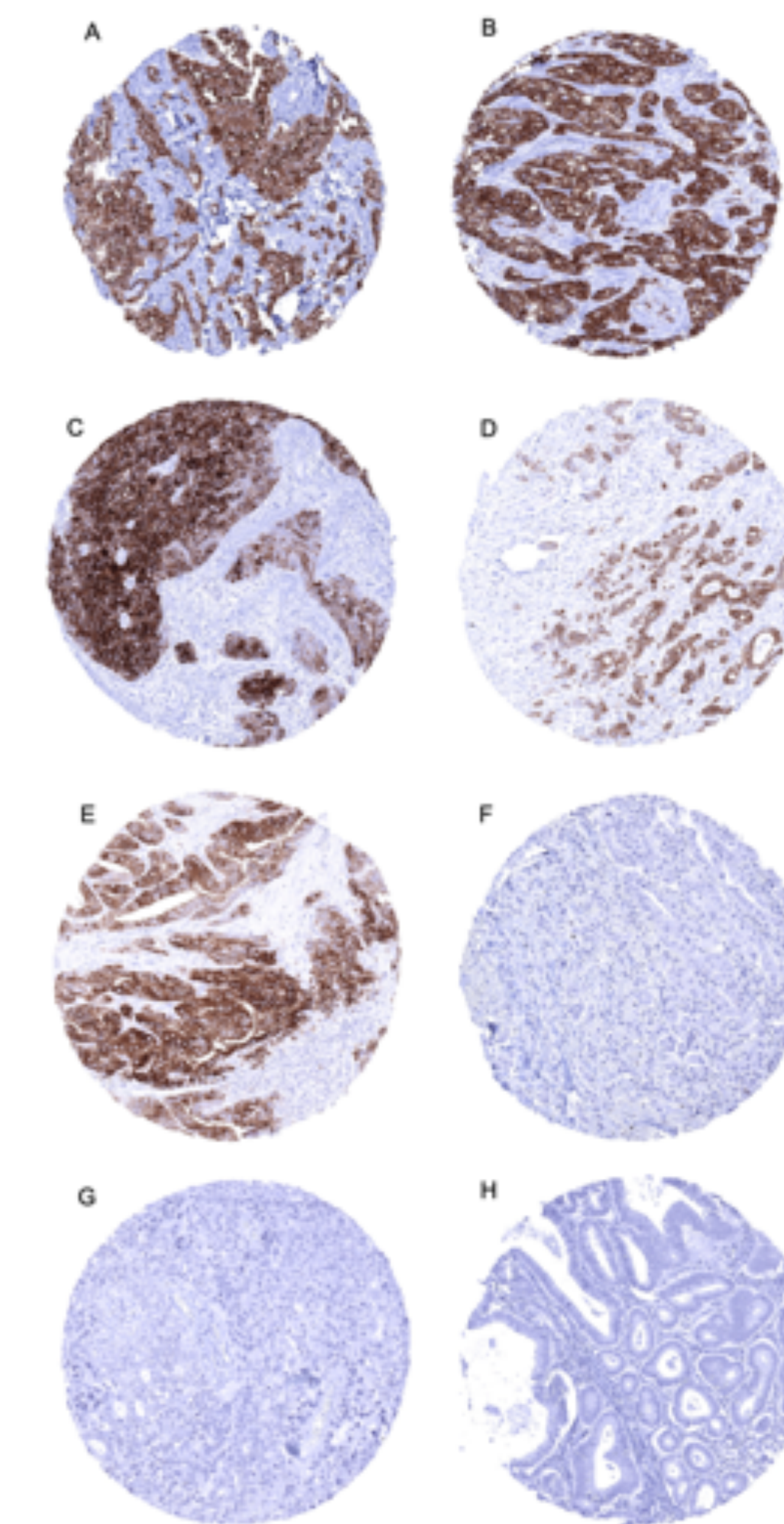


RESULTS

Tumor entity	n	Uroplakin 1B immunostaining				
		analyzable (%)	negative (%)	weak (%)	moderate (%)	strong (%)
Tumors of the skin						
Pilonid cyst	35	22	100.0	0.0	0.0	0.0
Basal cell carcinoma	88	74	98.6	1.4	0.0	0.0
Benign nevus	29	22	100.0	0.0	0.0	0.0
Squamous cell carcinoma of the skin	90	79	87.8	8.9	1.3	0.0
Malignant melanoma	48	41	100.0	0.0	0.0	0.0
Malignant melanoma Lymph node metastasis	87	87	100.0	0.0	0.0	0.0
Merkel cell carcinoma	46	35	100.0	0.0	0.0	0.0
Tumors of the head and neck						
Squamous cell carcinoma of the larynx	110	86	62.8	10.5	8.1	18.6
Squamous cell carcinoma of the pharynx	60	60	70.0	15.0	8.3	6.7
Oral squamous cell carcinoma (floor of the mouth)	130	122	82.0	7.4	4.9	5.7
Pleomorphic adenoma of the parotid gland	30	40	100.0	0.0	0.0	0.0
Wartin tumor of the parotid gland	49	43	34.9	53.5	7.0	4.7
Basal cell adenoma of the salivary gland	15	11	100.0	0.0	0.0	0.0
Tumors of the lung, pleura and thymus						
Adenocarcinoma of the lung	196	117	93.2	6.0	0.0	0.0
Squamous cell carcinoma of the lung	80	41	61.0	7.3	17.1	14.6
Small cell carcinoma of the lung	16	11	100.0	0.0	0.0	0.0
Mesothelioma, epithelioid	39	23	13.0	8.7	13.0	65.2
Mesothelioma, other types	76	43	55.8	16.3	7.0	20.9
Thymoma	29	24	96.8	4.2	0.0	0.0
Tumors of the female genital tract						
Squamous cell carcinoma of the vagina	78	49	87.8	10.2	2.0	0.0
Squamous cell carcinoma of the vulva	130	120	87.5	5.8	1.7	5.0
Squamous cell carcinoma of the cervix	130	121	89.3	7.4	1.7	1.7
Endometrioid carcinoma, high grade, G3	21	21	71.4	4.8	19.0	4.8
Endometrioid endometrial carcinoma	236	208	49.0	23.6	17.3	10.1
Endometrioid serous carcinoma	82	58	46.6	48.3	1.7	3.4
Carcinosarcoma of the uterus	48	31	67.2	22.6	6.5	3.2
Endometrial carcinoma, mixed type	13	12	69.2	25.0	7.7	6.3
Endometrial clear cell carcinoma	8	7	57.1	28.6	0.0	14.3
Endometrioid carcinoma of the ovary	110	83	54.2	32.5	9.6	3.6
Serous carcinoma of the ovary	559	302	42.3	43.3	8.2	0.6
Mucinous carcinoma of the ovary	96	69	65.2	21.7	11.6	1.4
Clear cell carcinoma of the ovary	50	35	68.6	14.3	14.3	2.9
Carcinosarcoma of the ovary	47	36	83.3	11.1	5.6	0.0
Gastricoid tumor of the ovary	37	100.0	0.0	0.0	0.0	0.0
Leydig cell tumor of the ovary	4	4	100.0	0.0	0.0	0.0
Sertoli cell tumor of the ovary	1	1	100.0	0.0	0.0	0.0
Sertoli Leydig cell tumor of the ovary	3	3	100.0	0.0	0.0	0.0
Sertoli cell tumor of the ovary	3	3	100.0	0.0	0.0	0.0
Brenner tumor	41	37	8.1	10.8	8.1	73.0
Tumors of the breast						
Invasive breast carcinoma of no special type	80	79	98.7	0.0	1.3	0.0
Lobular carcinoma of the breast	102	118	100.0	0.0	0.0	0.0
Medullary carcinoma of the breast	15	15	100.0	0.0	0.0	0.0
Tubular carcinoma of the breast	18	16	100.0	0.0	0.0	0.0
Mucinous carcinoma of the breast	22	20	100.0	0.0	0.0	0.0
Phyllodes tumor of the breast	50	46	100.0	0.0	0.0	0.0
Adenomatous polyp, low-grade dysplasia	50	42	100.0	0.0	0.0	0.0
Adenomatous polyp, high-grade dysplasia	50	47	100.0	0.0	0.0	0.0
Tumors of the digestive system						
Adenocarcinoma of the colon	2482	2117	99.3	0.6	0.0	0.1
Gastric adenocarcinoma, diffuse type	176	162	89.8	10.5	2.5	2.2
Gastric adenocarcinoma, intestinal type	174	163	99.8	6.1	3.1	0.0
Gastric adenocarcinoma, mixed type	62	58	89.7	6.9	3.4	0.0
Adenocarcinoma of the esophagus	83	77	83.1	9.1	7.8	0.0
Squamous cell carcinoma of the esophagus	75	70	74.7	12.9	7.1	6.7
Squamous cell carcinoma of the anal canal	91	78	89.7	6.4	1.3	2.6
Cholangiocarcinoma	114	104	77.9	10.6	7.7	3.8
Hepatocellular carcinoma	50	49	100.0	0.0	0.0	0.0
Ductal adenocarcinoma of the pancreas	612	464	70.3	17.7	9.3	2.8
Pancreatic/Ampullary adenocarcinoma	89	75	78.7	12.0	6.7	2.7
Acinar cell carcinoma of the pancreas	16	16	81.3	6.3	0.0	12.5
Gastrointestinal stromal tumor (GIST)	50	46	100.0	0.0	0.0	0.0
Non-invasive papillary urothelial carcinoma, pTa G2	177	128	4.7	8.6	26.6	60.2
Non-invasive papillary urothelial carcinoma, pTa G2	141	109	11.0	11.9	18.3	58.7
Non-invasive papillary urothelial carcinoma, pTa G3	219	176	13.1	21.6	19.3	46.0
Urothelial carcinoma, pT2-G3	739	640	9.6	14.2	12.2	32.0
Squamous cell carcinoma of the bladder	22	21	71.4	23.8	0.0	4.8
Small cell neuroendocrine carcinoma of the bladder	18	14	88.7	14.3	0.0	0.0
Sarcomatoid urothelial carcinoma	25	12	68.7	16.7	0.0	0.0
Urothelial carcinoma of the kidney pelvis	62	62	37.1	16.1	16.1	30.6
Clear cell renal cell carcinoma	858	793	91.4	6.9	1.4	0.3
Papillary renal cell carcinoma	255	238	53.4	34.9	10.5	1.3
Clear cell (tubul) papillary renal cell carcinoma	21	21	95.2	4.8	0.0	0.0
Chromophobe renal cell carcinoma	131	124	100.0	0.0	0.0	0.0
Oncocytoma	177	166	98.8	0.6	0.6	0.0
Tumors of the male genital organs						
Adenocarcinoma of the prostate, Gleason 3+3	83	83	100.0	0.0	0.0	0.0
Adenocarcinoma of the prostate, Gleason 4+4	80	80	99.8	1.3	0.0	0.0
Adenocarcinoma of the prostate, Gleason 5+5	85	84	100.0	0.0	0.0	0.0
Adenocarcinoma of the prostate (recurrence)	261	216	99.1	0.5	0.5	0.0
Small cell neuroendocrine carcinoma of the prostate	17	10	100.0	0.0	0.0	0.0
Seminoma	821	812	99.8	0.2	0.0	0.0
Embryonal carcinoma of the testis	50	46	100.0	0.0	0.0	0.0
Leydig cell tumor of the testis	30	30	100.0	0.0	0.0	0.0
Sertoli cell tumor of the testis	2	2	100.0	0.0	0.0	0.0
Sex cord stromal tumor of the testis	1	1	100.0	0.0	0.0	0.0
Spermatocytic tumor of the testis	1	1	100.0	0.0	0.0	0.0
Yolk sac tumor	50	46	97.8	2.2	0.0	0.0
Tumors of the endocrine organs						
Teratoma	50	40	92.5	5.0	2.5	0.0
Squamous cell carcinoma of the penis	80	80	93.8	3.8	1.3	1.3
Adenoma of the thyroid gland	114	100	100.0	0.0	0.0	0.0
Papillary thyroid carcinoma	392	263	96.5	1.5	0.0	0.0
Follicular thyroid carcinoma	158	127	99.2	0.8	1.2	0.0
Medullary thyroid carcinoma	107	97	100.0	0.0	0.0	0.0
Parathyroid gland adenoma	43	43	100.0	0.0	0.0	0.0
Anaplastic thyroid carcinoma	45	42	100.0	0.0	0.0	0.0
Adrenal cortical adenoma	50	48	100.0	0.0	0.0	0.0
Adrenal cortical carcinoma	26	25	100.0	0.0	0.0	0.0
Pheochromocytoma	50	48	100.0	0.0	0.0	0.0
Appendix, neuroendocrine tumor (NET)	22	12	100.0	0.0	0.0	0.0
Colorectal, neuroendocrine tumor (NET)	11	7	100.0	0.0	0.0	0.0
Ileum, neuroendocrine tumor (NET)	49	40	100.0	0.0	0.0	0.0
Lung, neuroendocrine tumor (NET)	19	17	94.1	5.9	0.0	0.0
Pancreas, neuroendocrine tumor (NET)	98	89	89.4	13.5	0.0	1.1
Colorectal, neuroendocrine carcinoma (NEC)	12	6	100.0	0.0	0.0	0.0
Gallbladder, neuroendocrine carcinoma (NEC)	4	3	100.0	0.0	0.0	0.0
Pancreas, neuroendocrine carcinoma (NEC)	14	14	100.0	0.0	0.0	0.0
Hodgkin Lymphoma	45	32	100.0	0.0	0.0	0.0
Tumors of soft tissue and bone						
Tenosynovial giant cell tumor	45	28	100.0	0.0	0.0	0.0
Granular cell tumor	53	32	100.0	0.0	0.0	0.0
Leiomyoma	50	50	100.0	0.0	0.0	0.0
Leiomyosarcoma	87	79	100.0	0.0	0.0	0.0
Liposarcoma	132	103	100.0	0.0	0.0	0.0
Malignant peripheral nerve sheath tumor (MPNST)	13	11	100.0	0.0	0.0	0.0
Myofibrosarcoma	26	26	100.0	0.0	0.0	0.0
Angiosarcoma	73	47	100.0	0.0	0.0	0.0
Angiomyolipoma	91	70	100.0	0.0	0.0	0.0
Dermatofibrosarcoma protuberans	21	14	100.0	0.0	0.0	0.0
Gangliocarcinoma	14	14	100.0	0.0	0.0	0.0
Kaposi sarcoma	8	1	100.0	0.0	0.0	0.0
Neurofibroma	117	110	100.0	0.0	0.0	0.0
Sarcoma, not otherwise specified (NOS)	75	67	100.0	0.0	0.0	0.0
Paraganglioma	41	41	100.0	0.0	0.0	0.0
Ewing sarcoma	23	12	100.0	0.0	0.0	0.0
Rhabdomyosarcoma	7	5	100.0	0.0	0.0	0.0
Schwannoma	121	116	100.0	0.0	0.0	0.0
Synovial sarcoma	12	8	100.0	0.0	0.0	0.0
Osteosarcoma	43	26	100.0	0.0	0.0	0.0
Chondrosarcoma	38	15	100.0	0.0	0.0	0.0
Rhabdoid tumor	5	5	100.0	0.0	0.0	0.0

UPK1b in cancers

Table 1: List of all tumor samples analyzed for Uroplakin 1B.



Upk1b immunostaining in cancer. The panels show a moderate to strong Upk1b positivity in cases of epithelioid malignant mesothelioma (A), muscle-invasive urothelial carcinoma (B), squamous cell carcinoma of the larynx (C), ductal adenocarcinoma of the pancreas (D), and an esophageal adenocarcinoma (E). Upk1b staining is absent in adenocarcinomas of the lung (F), the prostate (Gleason 5+5=10; G), and of the colorectum (H).

Positive staining for UPK1B was found in 61 (48%) different tumor types. At least one moderately positive tumor was seen in 50 (39%) tumor types, and 39 tumor types (31%) had at least one strongly positive tumor. Most frequent and strongest expression was found in urothelial neoplasms (58-95%), Brenner tumors of the ovary (92%), epithelioid mesothelioma (87%), serous carcinomas of the ovary (58%) and the endometrium (53%) as well as squamous cell carcinomas of various sites of origin (Table 1). UPK1B staining was rare in lung adenocarcinoma (6.8%) and largely absent in colorectal (0.7%) or prostatic adenocarcinoma (1.3%). In urothelial tumors cancer, low Upk1b expression was linked to high grade and invasive tumor growth (p<0.0001 each) as well as nodal metastasis (p=0.0006, Table 2).

Table 2: Uroplakin 1B expression and pathological features in urinary bladder cancer.

	Tumor stage	pTa G2 low	pTa G2 high	pTa G3	pT2	pT3	pT4	Uroplakin 1B immunostaining				P	
								n	negative (%)	weak (%)	moderate (%)		strong (%)
Urinary bladder cancer	Tumor stage	pTa G2 low	pTa G2 high	pTa G3	pT2	pT3	pT4	128	4.7	8.6	26.6	60.2	<0.0001
								109	11.0	11.9	18.3	58.7	
		144	14.6	20.1	22.2	43.1							
		139	42.4	15.1	10.8	31.7							
	Nodal												