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Prevalence of βIII-tubulin (TUBB3) expression in human normal tissues and cancers

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Abstract

Microtubules are multifunctional cytoskeletal proteins that are involved in crucial cellular roles including maintenance of cell shape, intracellular transport, meiosis, and mitosis. Class III beta-tubulin (βIII-tubulin, also known as TUBB3) is a microtubule protein, normally expressed in cells of neuronal origin. Its expression was also reported in various other tumor types, such as several types of lung cancer, ovarian cancer, and esophageal cancer. TUBB3 is of clinical relevance as overexpression has been linked to poor response to microtubule-targeting anti-cancer drugs such as taxanes. To systematically investigate the epidemiology of TUBB3 expression in normal and neoplastic tissues, we used tissue microarrays for analyzing the immunohistochemically detectable expression of TUBB3 in 3911 tissue samples from 100 different tumor categories and 76 different normal tissue types. At least 1 tumor with weak expression could be found in 93 of 100 (93%) different tumor types, and all these 93 entities also had at least 1 tumor with strong positivity. In normal tissues, a particularly strong expression was found in neurons of the brain, endothelium of blood vessels, fibroblasts, spermatogenic cells, stroma cells, endocrine cells, and acidophilic cells of the pituitary gland. In tumors, strong TUBB3 expression was most frequently found in various brain tumors (85%–100%), lung cancer (35%–80%), pancreatic adenocarcinoma (50%), renal cell carcinoma (15%–80%), and malignant melanoma (77%). In summary, these results identify a broad spectrum of cancers that can at least sporadically express TUBB3. Testing of TUBB3 in cancer types eligible for taxane-based therapies could be helpful to identify patients who might best benefit from this treatment.

Keywords

βIII-tubulin, multitumor tissue microarray, normal tissue, expression, immunohistochemistry

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Introduction

Microtubules are components of the cytoskeleton formed due to polymerization of dimeric structures composed of alpha- and beta-tubulins playing crucial roles in normal and cancerous cells including maintenance of cell shape, intracellular transport, meiosis, and mitosis.[1] They are dynamical structures that undergo continual assembly and disassembly in a cell.[2] Beta-tubulins exist as multiple isotypes with a complex pattern of distribution among different tissues.[3] Isotypes have been divided into several classes with distinct structural properties. Class III beta-tubulin (βIII-tubulin, encoded by the TUBB3 gene) is normally expressed at high levels in cells of neuronal origin but has

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also occasionally been found in several extra neuronal tissues including testes, placenta, small intestine, and colon.[3]

Aberrant expression of TUBB3 has been reported from a multitude of human cancer types, such as malignant melanoma,[4] non–small cell carcinoma,[4] adenocarcinoma of the colon,[5] ductal adenocarcinoma of the pancreas,[6] renal cell papillary carcinoma,[7] medulloblastoma, and oligodendroglioma[8] and has been linked to adverse tumor phenotype and poor clinical outcome in some of them.[7,9]

Alteration of TUBB3 in human cancers is of considerable clinical interest, because it has been linked to reduced response to taxane- and epothilone-based therapies.[10,11] For example, poor efficacy of microtubule-targeting anticancer drugs was reported from cancers with TUBB3 overexpression including non–small cell lung cancer,[12] uterine serous carcinoma,[11] advanced gastric cancer,[13] breast cancer,[13,14] and ovarian cancer.[15]

Tubulin-binding drugs inhibit microtubule dynamics required for DNA segregation and cell division, and, as a result, kill cancerous cells.[16] As the number of studies suggesting biological and clinical relevance of βIII-tubulin is rapidly increasing, there are also a growing number of reports showing considerable discrepancies with respect to the frequency of expression in various cancer types. For example, reported frequencies on βIII-tubulin expression ranges from 17% to 85% in non–small cell lung cancer,[17,18] from 20% to 67% in serous ovary cancer,[15,19] from 10% to 100% in colon adenocarcinoma,[5] and from 12% to 100% in prostate cancer.[19,20] These discrepancies are obviously due to the use of different antibodies and staining protocols in these studies.

The optimal approach for assessing the relative importance of a potentially relevant molecule across human tumor types includes the analysis of as large as possible number of different cancer types and subtypes together with a systematic evaluation of corresponding normal tissues. Moreover, it would be necessary to ensure a maximal standardization of all these analyses. The tissue microarray (TMA) technology is a suitable tool for such studies, as a large number of tissues can be analyzed on few TMA sections that are cut and stained in one day and in one set of reagents at completely identical staining conditions.

In this study, we took advantage of a set of preexisting TMAs containing more than 3900 samples from 100 different tumor types and subtypes as well as from 67 different normal tissues including more than 600 normal cell types. These tools enabled us to generate a comprehensive report on the frequency and levels of TUBB3 expression across tumor entities and normal cell types.

Materials and methods

Tissues

Two different sets of TMAs made from surgically removed organ parts, which were formalin-fixed and paraffin-embedded tissue samples, were used to study βIII-tubulin expression. The first was a normal TMA: Healthy tissue taken from organs operated for non-tumor reasons were composed of 8 samples each of 76 different normal tissue types (608 samples on one slide). The second TMA contained 3–92 (total: 3911) samples each from 100 different human tumor types and subtypes.[21] The samples of this second TMA were distributed among 8 different TMA blocks each containing between 454 and 532 samples. The exact composition of the TMA is given in the Results section. For both the TMA sets, tissue cylinders with a diameter of 0.6 mm were punched from tumor areas of each tissue block and brought into a recipient paraffin block using the paraffin sectioning aid system (adhesive-coated slides (PSA-CS4x), adhesive tape, and ultraviolet (UV) lamp; Instrumedics, Inc., Hackensack, NJ, USA). All tumor samples represented in these TMAs were obtained from the archives of the Institute of Pathology of the University Medical Center Hamburg-Eppendorf. Archived diagnostic leftover tissues were used to prepare TMAs and analyzed as approved by the local ethics committee (Ethics commission Hamburg, WF-049/09 and PV3652). According to local laws (HmbKHG, §12,1) informed consent was not necessary. All work has been carried out in compliance with the Helsinki Declaration.

Immunohistochemistry

Freshly cut TMA sections were stained on one day and in one experiment. Primary antibody specific for TUBB3 (clone EPR1568Y rabbit monoclonal antibody; dilution 1:150; Abcam, Cambridge, MA, USA) was added; slides were deparaffinized by 3× xylene washings for 3 min and 100%, 96%, 80% ethanol washings for 3 min; and exposed to heat-induced antigen retrieval for 5 min in an autoclave at 121°C in Tris-ethylenediaminetetraacetic acid (EDTA)-citrate buffer (pH 7.8). Bound antibody was then visualized using the EnVision detection system Kit K5007 Peroxidase/DAB+, rabbit/mouse (Dako, Glostrup, Denmark). Staining in nerves and axons served as a positive control. The pathologist recorded staining intensity (0, 1+, 2+, 3+) and fraction of positive tumor cells for each tissue spot. A final score was built from these two parameters according to the following criteria: negative scores had staining intensity of 0; weak ones had a staining intensity of 1+ in ≤70% of tumor cells or 2+ in ≤30% of tumor cells; moderate had staining intensity of 1+ in >70% of tumor cells, staining intensity of 2+ in >30% and ≤70% of tumor cells, or staining intensity of 3+ in ≤30% of tumor cells; and strong scores had staining intensity of 2+ in >70% of tumor cells or staining intensity of 3+ in >30% of tumor cells.[22]

Results

βIII-tubulin protein expression in normal tissues

The TUBB3 positive cell types in normal tissues are summarized in Table 1. Examples of TUBB3 positive normal tissues are given in Figure 1 and Figure S1.

Table 1. Expression of TUBB3 in human normal tissues.

Organ	Cell type
Skin, epidermis	Basal cell++, melanocytes+++
Skin, sebaceous glands	Peripheral germinative cell
Peripheral nerves	Perineural cell++
Aorta, intima	Endothelium++
Aorta, media	Endothelium++
Heart	Myocytes++, endothelium++
Striated muscle	Endothelium++
Tongue, muscle	Neurons+++, endothelium++
Uterus, myometrium	Endothelium++, muscular cell+
Appendix, muscular wall	Neuron+++
Esophagus, muscular wall	Endothelium++
Stomach, muscular wall	Neurons+++, endothelium++
Ileum, muscularis	Endocrine cells+++, fibroblasts++
colon descendens, muscular wall	Endothelium++, neuron+++
Kidney pelvis	Endothelium++, fibroblasts++
Urinary bladder, muscular wall	Neuron+++, endothelium++
Penis glans	Neurons+++, endothelium++
Ovary, stroma	Neurons++, stroma+++
Fat	–
Lip, oral muosa	Basal cells++, melanocytes+++
Oral cavity	Basal cells++
Tonsil, surface epithelium	Basal cells++, germinal centers+++
Anal canal, skin	Basal cells++, melanocytes+++, keratinocytes+
Anal canal, transitional mucosa	Basal cells++, melanocytes+++, keratinocytes+
Ectocervix	Basal cells++
Esophagus, squamous epithelium	Basal cells++, keratinocytes+
Kidney, pelvis, urothelium	Basal cells++, intermediate cells++, superficial cells++
Urinary bladder, urothelium	Basal cells++, intermediate cells++, superficial cells++
Placenta, mature, amnion and chorion	Mesenchymal cells+++, endothelial cell fetal capillaries
Lymph node	–
Spleen	Endothelium+++
Gall bladder, epithelium	Endothelium++, Fibroblasts++
Liver	Endothelium++
Pancreas	Pancreatic islets+++, endothelium++, fibroblasts++
Parotid gland	Fibroblasts++
Appendix, mucosa	Germinal centers+++, fibroblasts+++, neurons+++, endothelium+++, endocrine cells+++
Rectum, mucosa	Endocrine cells+++, fibroblasts+++, neurons+++, apocrine cells+
Colon descendens, mucosa	Endothelium++
Ileum, mucosa	Endothelium++, fibroblasts++, endocrine cells+++
Duodenum, mucosa	Endothelium++, fibroblasts++, endocrine cells+++
Stomach, corpus	Endothelium+, fibroblasts++, endocrine cells+++
Stomach, antrum	Endothelium+, fibroblasts++, endocrine cells+++
Tonsil	Germinal centers+++
Thymus	Hassall's corpuscles
Submandibular gland	Endothelium++
Sublingual gland	Neurons+++, columnar duct cell+++
Bone marrow	–
Duodenum, Brunner's gland	–
Kidney, cortex	Distal tubulus epithelium
Kidney, medulla	–
Prostate	Secretory cells+, fibroblasts+
Seminal vesicle	Columnar duct cells++
Epididymis	Tall slender cell+
Testis	Spermatogonic cells+++, primary spermatocytes+, secondary spermatocytes++, sertoli cells++

(continued)

Table 1. (continued)

Organ	Cell type
Bronchus, mucosa	Basal cell+, neuroendocrine cells++
Bronchus, glands	Endothelium++
Sinus paranasales	Ciliated cells++
Lung	Endothelium++, macrophages+
Breast	Fibroblasts+
Endocervix	Endothelium++, fibrocytes++, adrenaline
Endometrium, proliferation	Stroma cells+++, epithelium+++
Endometrium, secretion	Stroma cells+++, epithelium+++
Fallopian tube, mucosa	Ciliary cells+
Placenta early, decidua	Decidual cells+++, endothelium++
Ovary, corpus luteum	Granulosa lutein cells++, theca interna++
Ovary, follicular cyst	Endothelium++, theca interna+
Placenta, early	Syncytiotrophoblast+++, endothelium++, neuroendocrine cell++, trophoblast+
Placenta, mature	Syncytiotrophoblast++, endothelium++
Adrenal gland	Pheocromocyte cells+++, cortical cells+++, sustentacular cells+++
Parathyroid	–
Thyroid gland	–
Cerebellum, cortex	Neurons+++, nerve fibers+++
Cerebellum, gray	Purkinje cell+++, neurons+++
Cerebrum, gray	Neuron+++, nerve fibers+++
Cerebrum, white	Nerve fiber+++
Pituitary, posterior lobe	Cidophilic cells+++, basophilic cells+++, chromophobic cells+++
Pituitary, anterior lobe	Acidophilic cells+++, basophilic cells+++, chromophobic cells+++

–: no detectable staining; +: weak staining; ++: moderate staining; +++: strong staining.

βIII-tubulin protein expression in tumors

βIII-tubulin expression was analyzable in at least 1 sample in 99 of the 100 arrayed tumor types. No result could be obtained from chrondrosarcomas, because the tissue spots were exhausted or lacked sufficient numbers of tumor cells. At least a weak βIII-tubulin protein expression could be detected in virtually all (93 of 98, 95%) of the analyzable tumor categories, all of which also contained at least one tumor spot with strong positivity. The immunohistochemical results are given in Table 2 for all tumor types having at least one positive case. βIII-tubulin positivity was most striking in neuronal tumors, including oligodendrogliomas and neuroblastomas, where all examined tumors showed strong positivity. As expected, other neuronal tumors, such as astrocytomas and pheochromocytomas, were also among the most frequently positive tumors. Frequent strong βIII-tubulin protein positivity was further seen in medullary thyroid cancers (17/20, 85%), malignant mesotheliomas (11/13, 85%), Hodgkin's lymphoma (10/13, 77%), seminomas (44/66, 67%), and small cell lung cancers (8/10, 80%). Representative image of tumor types with strong βIII-tubulin positivity are shown in Figure 1. Tumor types without detectable βIII-tubulin protein expression under the selected experimental conditions included non-Hodgkin's lymphomas (0 of 5), adenomas of the thyroid gland (0 of 48), lymphoepithelial carcinomas (0 of 1), leiomyomas (0 of 16), and haemangiopericytomas (0 of 4). However, the number of examined cases of several of these tumor types is low. These data therefore do not rule out that βIII-tubulin expression can sometimes also occur in these tumor types.

Discussion

The study shows that high levels of βIII-tubulin protein expression are seen in a large variety of cancer types and subtypes suggesting that this protein may have considerable general importance in cancer biology. There were 66 tumor types that were newly identified as having at least an occasional βIII-tubulin protein overexpression in this study. These included many important cancer types such as squamous cell carcinoma of the skin, squamous cell carcinoma of the vulva and vagina, gastrointestinal stroma tumor (GIST), adenocarcinoma of the gall bladder, papillary adenocarcinoma of the pancreas, neuroendocrine carcinoma of the pancreas, seminoma, urothelial carcinoma, thyroid cancer, neuroblastoma, desmoid-tumor, and stroma-sarcoma.

A protein may have a particularly strong role for cancer biology in tumors where the target expression is substantially higher or lower than in its corresponding normal tissues. Such a result was found for overexpression in lung, thyroid gland, kidney, gastrointestinal tissues, and the breast. In particular, βIII-tubulin protein was overexpressed in lung cancer, breast cancer, thyroid cancer, squamous cell carcinoma of the skin, and basalioma, while lung tissue, thyroid tissue, and skin epithelium were largely βIII-tubulin protein negative. A significant role of βIII-tubulin protein in lung

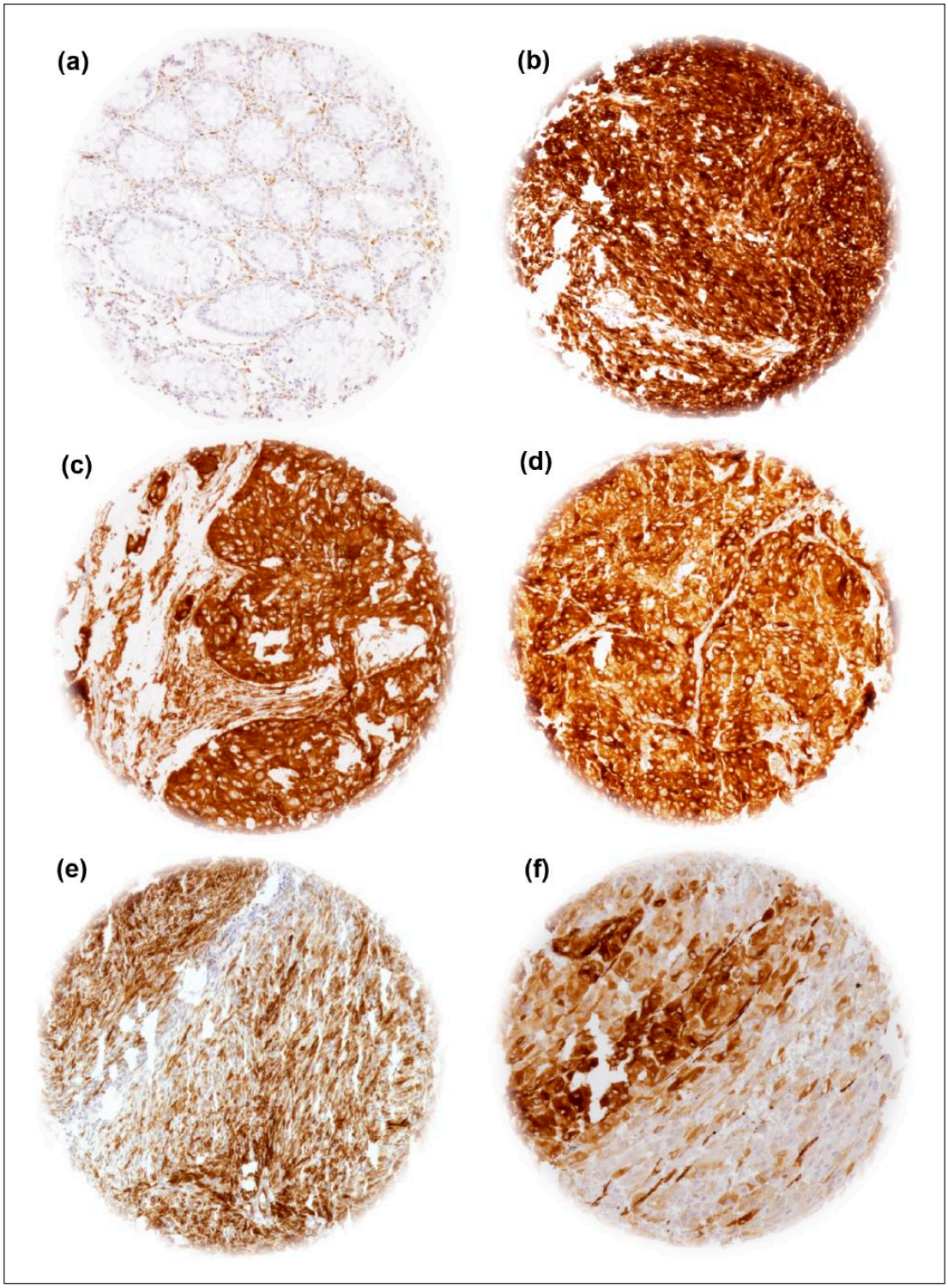

Figure 1. Representative images of TUBB3 staining in normal tissues and tumors: (a) normal colon, TUBB3 staining in endothelial cells and neurons, (b) gastrointestinal stromal tumor (GIST), (c) esophageal adenocarcinoma, (d) gall bladder cancer, (e) basalioma, and (f) malignant melanoma.

cancer,[12] ovarian cancer,[15,19,23,24] and breast cancer[19] was already suggested demonstrating an independent prognostic impact in malignant melanoma,[25] ovarian cancer,[26] and non–small cell lung cancer.[27] Moreover, Zhang et al.[28] had shown that TUBB3 might be used as a prognostic marker for biomarker-guided chemotherapy in non–small cell lung cancer. A comparison of the results of earlier studies on TUBB3 expression with our own study is given in Figure 2.

Table 2. Expression of TUBB3 in human cancers.

Tumor type	n	TUBB3 IHC result (%)			
		Negative	Weak	Moderate	Strong
Skin tumor					
Merkel cell cancer	2	0.0	0.0	0.0	100.0
Basal cell adenoma	29	13.8	3.4	20.7	62.1
Nevus, benign	15	20.0	6.7	0.0	73.3
Malignant melanoma	22	22.7	0.0	9.1	68.2
Skin cancer, SqCC	33	30.3	3.0	0.0	66.7
Respiratory tract tumor					
Malignant mesothelioma	13	15.4	0.0	0.0	84.6
Lung cancer, small cell cancer	10	20.0	0.0	0.0	80.0
Lung cancer, adenocarcinoma	50	32.0	0.0	2.0	66.0
Lung cancer, large cell cancer	21	33.3	0.0	0.0	66.7
Larynx, carcinoma	34	41.2	2.9	17.6	38.2
Lung cancer, SqCC	51	49.0	0.0	3.9	47.1
Lung cancer, NSCLC	2	50.0	0.0	0.0	50.0
Lung cancer, bronchioalveolar carcinoma	14	64.3	0.0	0.0	35.7
Gastrointestinal tumor					
Oral cavity, carcinoma	40	15.0	2.5	30.0	52.5
Carcinosarcoma	26	15.4	0.0	7.7	76.9
Pancreatic cancer, neuroendocrine	14	21.4	0.0	0.0	78.6
GIST	37	21.6	2.7	16.2	59.5
Esophageal carcinoma, SqCC	41	24.4	4.9	36.6	34.1
Gall bladder carcinoma	16	31.3	0.0	0.0	68.8
Esophageal carcinoma, adenocarcinoma	37	32.4	5.4	16.2	45.9
Small intestine carcinoma	13	38.5	0.0	15.4	46.2
Colon cancer	31	48.4	0.0	6.5	45.2
Pancreatic cancer, ductal adenocarcinoma	28	50.0	0.0	0.0	50.0
Colon adenoma, high grade	14	50.0	7.1	21.4	21.4
Pancreatic cancer, papillary adenoma	12	58.3	0.0	8.3	33.3
Parotid, pleomorphic adenoma	51	58.8	0.0	5.9	35.3
Colon adenoma, low grade	24	66.7	4.2	8.3	20.8
Anal carcinoma	6	66.7	0.0	0.0	33.3
Stomach cancer, diffuse type	3	66.7	0.0	0.0	33.3
Basalioma	28	71.4	3.6	3.6	21.4
Warthin's tumor	44	72.7	0.0	20.5	6.8
Mucoepidermoid carcinoma	24	75.0	0.0	8.3	16.7
Stomach cancer, intestinal type	25	80.0	0.0	0.0	20.0
Hepatocellular carcinoma	41	95.1	0.0	0.0	4.9
Lymphoepithelial tumor	1	100.0	0.0	0.0	0.0
Gynecological tumor					
Cervical cancer, adenosquamous carcinoma	1	0.0	0.0	0.0	100.0
Endometrial cancer, serous carcinoma	34	20.6	0.0	0.0	79.4
Vulva carcinoma, SqCC	40	37.5	0.0	2.5	60.0
Ovarian cancer, serous carcinoma	52	38.5	5.8	11.5	44.2
Breast cancer, mucinous carcinoma	38	39.5	0.0	0.0	60.5
Ovarian cancer, endometroid carcinoma	15	40.0	6.7	0.0	53.3
Breast cancer, medullary carcinoma	46	50.0	2.2	2.2	45.7
Breast cancer, ductal carcinoma	38	52.6	2.6	5.3	39.5
Vagina carcinoma, SqCC	11	54.5	0.0	0.0	45.5
Cervical cancer, SqCC	51	60.8	0.0	3.9	35.3
Endometrial cancer, endometroid carcinoma	54	61.1	0.0	3.7	35.2
Ovarian cancer, mucinous carcinoma	34	64.7	0.0	2.9	32.4
Breast cancer, apocrine carcinoma	12	66.7	0.0	0.0	33.3
Breast cancer, tubulary carcinoma	25	68.0	0.0	0.0	32.0
Breast cancer, cribriform carcinoma	13	69.2	0.0	7.7	23.1
Breast cancer, phylloid carcinoma	20	90.0	0.0	0.0	10.0

(continued)

Table 2. (continued)

Tumor type	n	TUBB3 IHC result (%)			
		Negative	Weak	Moderate	Strong
Breast cancer, lobulary carcinoma	32	90.6	0.0	0.0	9.4
Genitourinary tract tumor					
Testis, seminoma	66	6.1	3.0	9.1	81.8
Renal cell cancer, papillary	14	21.4	0.0	7.1	71.4
Urinary bladder cancer, nos	7	28.6	0.0	0.0	71.4
Urinary bladder cancer, invasive (pT2–4)	42	31.0	4.8	9.5	54.8
Renal cell cancer, nos	3	33.3	0.0	0.0	66.7
Penile carcinoma	28	39.3	0.0	17.9	42.9
Testis, non-seminoma	27	44.4	3.7	11.1	40.7
Oncocytoma	16	62.5	25.0	6.3	6.3
Renal cell cancer, chromophobic	32	68.8	3.1	3.1	25.0
Urinary bladder cancer, non-invasive (pTa)	54	70.4	7.4	11.1	11.1
Teratoma	26	80.8	0.0	3.8	15.4
Cervical cancer, adenocarcinoma	25	84.0	0.0	8.0	8.0
Prostate cancer	34	85.3	8.8	2.9	2.9
Renal cell cancer, clear cell	38	86.8	2.6	0.0	10.5
Ovarian cancer, Brenner tumor	25	96.0	0.0	0.0	4.0
Neuronal tumor					
Neuroblastoma	41	0.0	0.0	0.0	100.0
Oligodendroglioma	20	0.0	0.0	0.0	100.0
Medulloblastoma	4	0.0	0.0	0.0	100.0
Astrocytoma	32	0.0	0.0	3.1	96.9
Ependymoma	7	14.3	0.0	0.0	85.7
Malignant schwannoma	9	66.7	0.0	0.0	33.3
Neuroendocrine tumor					
Thyroid carcinoma, anaplastic	1	0.0	0.0	0.0	100.0
Pheochromocytoma	54	1.9	0.0	1.9	96.3
Paraganglioma	25	4.0	0.0	0.0	96.0
Adrenal cortex, carcinoma	7	14.3	0.0	14.3	71.4
Thyroid carcinoma, medullary	20	15.0	0.0	0.0	85.0
Carcinoid	18	22.2	0.0	16.7	61.1
Thyroid carcinoma, papillary	44	52.3	2.3	11.4	34.1
Adrenal cortex, adenoma	19	63.2	0.0	21.1	15.8
Thyroid carcinoma, follicular	38	92.1	0.0	2.6	5.3
Thyroid, adenoma	48	100.0	0.0	0.0	0.0
Soft tissue tumor					
Desmoid tumor	7	0.0	0.0	0.0	100.0
Granular cell cancer	3	0.0	0.0	0.0	100.0
Dermatofibrosarcoma protuberans	1	0.0	0.0	100.0	0.0
Giant cell tumor of the tendon sheat	13	7.7	0.0	0.0	92.3
Pilomatrixoma	15	13.3	0.0	6.7	80.0
Stroma sarcoma	3	33.3	0.0	0.0	66.7
Leiomyosarcoma	27	51.9	0.0	0.0	48.1
Malignant fibrous histiocytoma	18	55.6	0.0	0.0	44.4
Angiosarcoma	3	66.7	0.0	0.0	33.3
Liposarcoma	13	84.6	0.0	0.0	15.4
Neurofibroma	30	90.0	0.0	0.0	10.0
Hemangiopericytoma	4	100.0	0.0	0.0	0.0
Leiomyoma	16	100.0	0.0	0.0	0.0
Hematological neoplasia					
Thymoma	39	82.1	0.0	2.6	15.4
Non-Hodgkin's lymphoma	5	100.0	0.0	0.0	0.0
Hodgkin's lymphoma	13		23.1	0.0	61.5

IHC: immunohistochemistry; n: number of analyzed samples; SqCC: squamous cell carcinoma; NSCLC: non–small cell lung cancer; GIST: Gastrointestinal stroma tumor; nos: not otherwise specified.

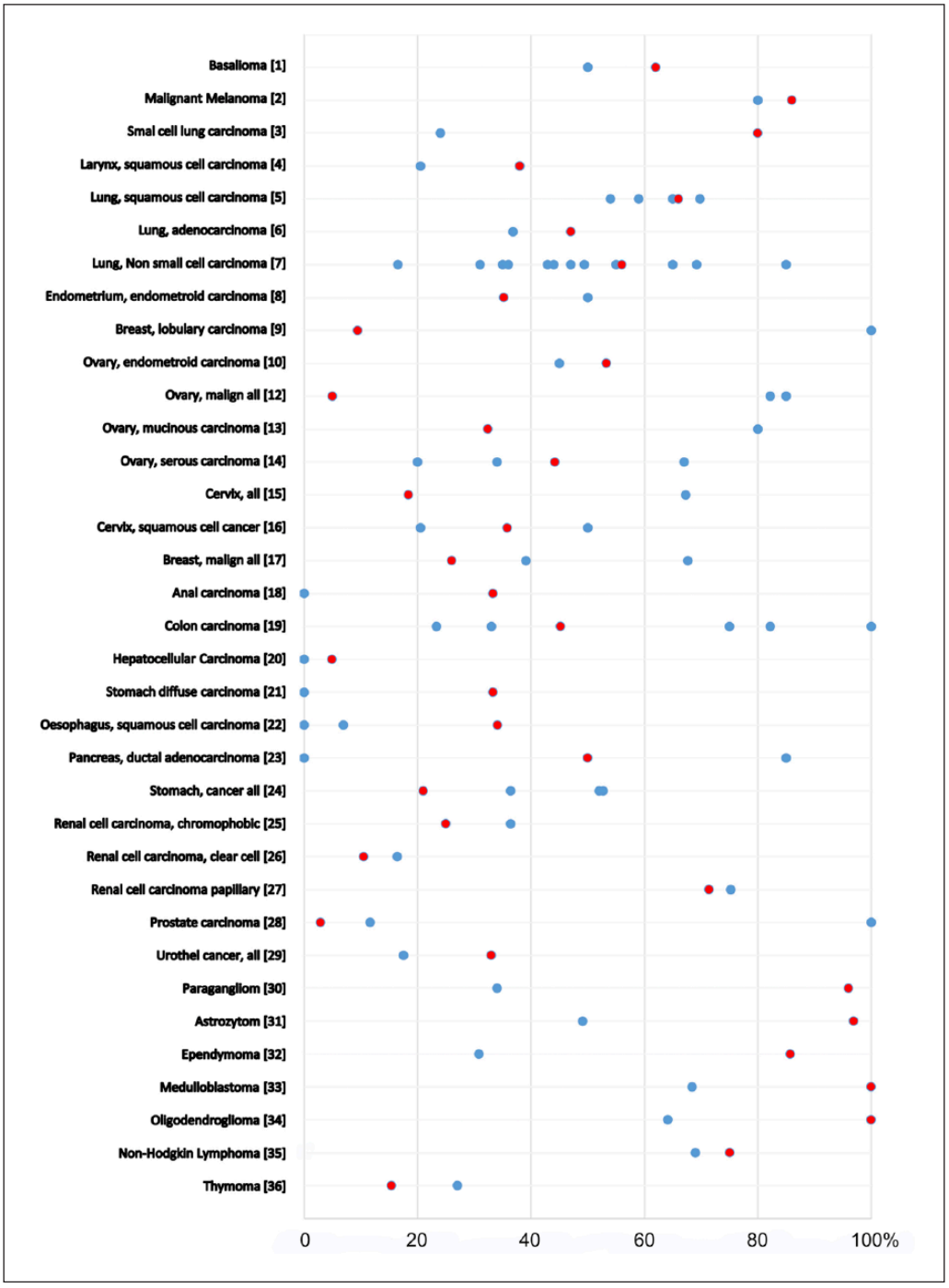

Figure 2. Percentage of TUBB3 cancers in previous studies (blue dots) as compared to the results of our study (red dots). Figures [1],[29] [2],[4] [3],[30] [4],[31,32] [5],[32,33] [6],[33] [7],[5,12,18,34–42] [8],[19] [9],[19] [10],[15] [12],[15] [13],[15] [14],[15,19,43] [15],[44] [16],[19,31] [17],[45,46] [18],[19] [19],[5,19,47–49] [20],[50] [21],[19] [22],[19,51] [23],[6,19] [24],[52–54] [25],[7] [26],[7] [27],[7] [28],[19,20] [29],[55] [30],[56] [31],[8] [32],[8] [33],[8] [34],[8] [35],[54,57] and [36].[39]

Importantly, TUBB3 showed marked expression differences between tumor types that are sometimes difficult to diagnose based on histological examination alone. This was most evident for thyroid gland lesion, where benign adenomas and carcinomas share similar histological features. Under the selected experimental conditions, TUBB3 staining was absent in thyroid gland adenomas (0/48) but present in a relevant fraction of thyroid papillary carcinomas (21/44, 48%) and thyroid follicular carcinomas (3/38, 8%). TUBB3 immunohistochemistry might thus be helpful to secure the diagnosis of thyroid carcinoma in case of positive staining. Potentially relevant differences in the TUBB3 expression frequency were also seen between papillary (11/14, 79%) and clear cell kidney cancers (5/38, 13%). This finding is in

agreement with an earlier study on TUBB3 expression in a larger number of renal cell cancers by our group,[7] demonstrating that the results from the comparatively small numbers of samples per cancer type in this study are largely representative for the analyzed cancer types.

TUBB3 is of high clinical importance because overexpression has been linked to reduced response to taxane- or epithelione-based therapies.[10,11] In fact, all cancer types with reported poor response to these drugs also showed high level and frequent TUBB3 staining in our TMA study, including, for example, non–small cell lung cancers,[12] breast,[13,14] bladder,[55] prostate,[19,20] esophageal,[51] testicular germ cell tumors,[58] penile cancer,[59] and ovarian cancers.[15] On the other end of the spectrum, our study identified hepatocellular carcinoma as a cancer type showing only occasional (5%) TUBB3 expression. Several in vitro studies have suggested that liver cancers may respond well to taxanes,[60] but the clinical value of taxanes in liver cancer patients is poorly understood. So far, only a small phase II study on 20 patients with unresectable liver cancers was undertaken but did not show a benefit from paclitaxel therapy.[33] The findings of our study may encourage further research on this topic. Our analysis provides an almost complete overview on βIII-tubulin protein expression in normal and neoplastic human tissues. TMAs are an ideal tool to massively accelerate characterization of novel biomarkers. The use of TMAs to screen normal tissues and different tumor types for molecular alterations of interest is an obvious application of this technique. Earlier, we had used comparable multitumor TMAs for the evaluation of calretinin,[61] KIT[62] and ERG.[63] It is a distinct advantage of the TMA technique that all tissues are analyzed under maximally standardized conditions. Although automated immunostainers—despite some remaining day-to-day variability—can provide good standardization of the staining process, TMAs enable a control of several additional important parameters affecting staining. For example, the TMA method enables to cut the sections of TMA blocks containing samples from 5000 samples within 1 h. If these sections were taken from regular tissue blocks, sectioning was likely to last several weeks. Several studies have demonstrated, however, that immunoreactivity of tissue sections decreases markedly over time.[64–66] For most antibodies, storage of cut sections for 2 weeks already significantly impacts staining results.

In summary, the results of our study demonstrate that TUBB3 upregulation is a common feature that occurs at different frequencies in almost every type of cancer. Studies are needed to clarify whether TUBB3 testing in patients might predict response to microtubule-targeting anti-cancer drugs in a clinically relevant way.

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