IMP3 overexpression occurs in various important cancer types and is linked to aggressive tumor features: A tissue microarray study on 8,877 human cancers and normal tissues

CHRISTOPH BURDELSKI[1*], NILOFAR JAKANI-KARIMI[2*], FRANK JACOBSEN[2], CHRISTINA MÖLLER-KOOP[2], SARAH MINNER[2], RONALD SIMON[2], GUIDO SAUTER[2], STEFAN STEURER[2], TILL S. CLAUDITZ[2] and WALDEMAR WILCZAK[2]

[1]General, Visceral and Thoracic Surgery Department and Clinic, University Medical Center Hamburg-Eppendorf; [2]Institute of Pathology, University Medical Center Hamburg-Eppendorf, Hamburg, Germany

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Abstract. IMP3 is an RNA binding protein required for ribosomal RNA processing, which has been suggested to be a prognostic marker in a large variety of human types of cancer. However, available data on the prevalence of IMP3 expression are largely discrepant. To systematically investigate the epidemiology and clinical relevance of IMP3 expression in human cancers we employed a two-step tissue microarrays (TMAs) approach. First, a normal tissue TMA and a multi-tumor TMA were analyzed for immunohistochemically detectable expression of IMP3 in 76 different normal tissue types and 3889 cancer samples from 95 different tumor categories. In a second step, we searched for associations between IMP3 expression and tumor phenotype and patient prognosis in TMAs containing 697 urinary bladder cancers, 1711 colon cancers, 343 esophageal adenocarcinomas, 251 esophageal squamous cell cancers, 673 lung cancers), 275 pancreatic cancers and 230 stomach cancers. In normal tissues, unequivocal IMP3 expression was found in placenta, lymphocytes and some types of glandular epithelial cells. In cancers, at least one case with weak expression could be found in 76 out of 95 (80%) different tumor types and 64 entities (67%) had at least one tumor with strong positivity. IMP3 expression was most frequently found in testicular cancer (including 71% seminomas and 96% non-seminomas), neuroblastoma (88%), and squamous cell cancer of various origins. Significant associations were found between IMP3 and adverse tumor features in esophageal adenocarcinomas and cancers of the urinary bladder, lung, stomach, and pancreas. In summary, IMP3 was frequently expressed in many different tumor types, and was typically associated with aggressive tumor features.

Correspondence to: Dr Ronald Simon, Institute of Pathology, University Medical Center Hamburg-Eppendorf, Martinistrasse 52, D-20246 Hamburg, Germany
E-mail: r.simon@uke.de

*Contributed equally

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Introduction

Insulin-like growth factor II m-RNA-binding protein 3 (IMP3) is a member of the IMP family playing an important role in cell migration in early embryogenesis (1,2). This 'U3 small nucleolar ribonucleoprotein' is a component of an RNA binding protein required for the early cleavage during pre-18s ribosomal RNA processing. Previously, IMP3 has gained considerable interest as a cancer-associated protein. IMP3 overexpression has been reported in a variety of human types of cancer, including lung cancer (3), germ cell cancer (4), colon cancer (5), pancreatic cancer (6), gastric cancer (7), liver cancer (8), and kidney cancer (9), and has been linked to advanced disease stage and adverse clinical outcome in some of these cnacers (5,7,8,10-12). Collectively, these studies strongly suggest that IMP3 may represent a valuable prognostic marker in human cancer. However, as the number of studies suggesting biological and clinical relevance of IMP3 is rapidly increasing, there are also a growing number of reports revealing considerable discrepancies with respect to the frequency of expression in various types of cancer. For example, reported frequencies in IMP3 expression ranges from 0 to 83% in prostate cancer (13-16), from 11 to 86% in papillary thyroid cancer (17-20), from 11 to 65% in papillary renal cell cancer (9,12), from 0 to 52% in leiomyoma (21,22), from 21 to 71% in invasive urinary bladder tumor (23,24), from 50 to 100% in small cell lung cancer (3,25), and from 37 to 83% in malignant mesothelioma (26,27). Such discrepancies may be due to the use of different antibodies, staining protocols, and scoring criteria in these studies. The optimal study for assessing the relative importance of a potentially relevant molecule across tumor types includes the analysis of as large a number of different normal tissues, cancer types and subtypes as possible, followed by the evaluation of the clinical value of IMP3 in selected types of cancer with

frequent IMP3 expression. Moreover, it would be necessary to ensure a maximal standardization of all these analyses. Tissue microarray (TMA) technology is a suitable tool for such a study, as a large number of tissues can be analyzed on few sections that are cut in 1 day and that can be stained in 1 day in a set of reagents under completely identical staining conditions.

In this study, we utilized a two-step tissue microarray (TMA) approach to evaluate the clinical utility of IMP3 testing in human normal tissues and cancer. In a first step, we screened 76 different normal tissue types and samples of 95 different tumor types using a multi-tumor TMA. In a second step, tumor-type specific TMAs with clinical follow-up data were utilized to evaluate the clinical significance of IMP3 alterations in five selected tumor entities. Our approach implicated frequent IMP3 expression in 76 different tumor types and concomitantly demonstrated the association between IMP3 expression and poor prognosis in adenocarcinomas of the lung.

Materials and methods

Human tissues. The construction of tissue microarrays was as reviously described (28). The normal tissue TMA was composed of 8 samples each of 76 different normal tissue types (608 samples on one slide). The multi-tumor TMA contained 3,899 primary tumors from 95 different tumor types and subtypes distributed among 10 different TMA blocks each containing between 350 and 680 samples. The exact composition of this TMA is presented in Table I. In addition, six different prognosis TMAs were analyzed, representing 697 urinary bladder cancers (694 with clinical follow-up data), 1711 colon cancers (1709 with clinical follow-up data), 343 esophageal adenocarcinomas (300 with clinical follow-up data), 251 esophageal squamous cell cancers (244 with clinical follow-up data), 673 lung cancers (269 with clinical follow-up data), 275 pancreatic cancers (219 with clinical follow-up data), and 230 stomach cancers (146 with clinical follow-up data). The composition of these TMAs has been described before (29-33). No informed consent was obtained in accordance with the local law (HmbKHG, §12,1).

Immunohistochemistry. Freshly cut TMA sections were analyzed. IMP3 expression was detected with a monoclonal mouse anti-human antibody (clone 69.1; Dako M3626, Glostrup, Denmark) in a dilution of 1:100 after peroxidase blocking with H_2O_2 (Dako S2023) for 10 min. High-temperature pretreatment of slides was carried out in an autoclave with citrate buffer, pH 9.0 for 5 min. The Envision system (Dako 5007) was used to visualize the staining. In normal tissues, a cell type specific distribution of IMP3 expression was recorded, and the staining intensity was estimated as weak (+), moderate (++), or strong (+++). In tumor tissues, cytoplasmic staining was evaluated by staining intensity (0, 1+, 2+, and 3+), and the fraction of positive tumor cells was scored for each tissue spot. A final score was built from these two parameters according to the following criteria: negative scores had a staining intensity of 0 and 1+ in ≤10% of tumor cells; weak scores had a staining intensity of 1+ in >10% and ≤70% of tumor cells or a staining intensity of 2+ in ≤30% of tumor cells; moderate scores had a staining intensity of 1+ in >70% of tumor cells, a staining intensity of 2+ in >30% and ≤70% of tumor cells or a staining intensity of 3+ in ≤30% of tumor cells; and strong scores had a staining intensity of 2+ in >70% of tumor cells or a staining intensity of 3+ in >30% of tumor cells. All tumors exhibiting at least weak expression were defined as IMP3-positive.

Statistical analysis. For statistics, JMP® 12.0 software (SAS institute Inc., Cary, NC, USA) was used. All P-values were two-sided, and P-values <0.05 were considered as significant. To study the relationship between IMP3 expression and clinicopathological parameters, contingency table analysis and Chi-square test (likelihood) were used. Analysis on recurrence-free and overall survival was performed using the Kaplan-Meier method and was compared via log-rank test. Cox regression and multivariate analyses were used to assess independence of IMP3 staining.

Results

IMP3 protein expression in normal tissues. Positive IMP3 staining was seen in few normal tissues and in specific cell types only, including amnion (+), chorion cells (+) syncytiotrophoblast (+++), cytotrophoblast (+++), decidua (+) and mesenchymal cells (++) of the placenta, lymph follicles in lymph nodes and tonsils (lymphoblasts (+++), lymphocytes (+++), thymocytes (+), absorptive cells of the ileum (+++), crypt cells of rectal mucosa (+), mucus cells (++) of submandibular and sublingual glands, spermatogonia of the testis (++), ciliated cells (+) of bronchial mucosa, mucinous acinar cells of bronchial glands (++), secretory cells of the endocervix (+), ciliated cells of the fallopian tube (+++), and cells of the adenohypophysis of the anterior lobe of the pituitary gland (+).

Normal tissue samples that were also analyzed but did not exhibit any IMP3 staining included: aorta, heart, striated muscle, tongue, uterus, appendix, esophagus, stomach, ileum muscle, colon, kidney, urinary bladder, penis, ovary, fat, skin, lip, oral cavity, anal canal, ectocervix, spleen, duodenum, gallbladder, liver, pancreas, parotid gland, bone marrow, prostate, seminal vesicles, epididymis, nose sinus, lung, breast, adrenal gland, parathyroid gland, thyroid gland, cerebellum and cerebrum.

IMP3 protein expression in tumors. Analyzable results could be obtained from 96 of the 99 types of cancer represented in our multi-tumor TMA. At least weak IMP3 protein expression could be detected in 76 (80%) of the 95 tumor categories with analyzable results, including 64 (67%) categories where at least one tumor revealed a strong positivity. The immunohistochemical results are summarized in Table I. IMP3 positivity was most striking in testicular cancers, where all examined tumors exhibited positive staining, including 71% (seminomas) and 96% (non-seminomas) with strong staining. Additional cancers with frequent IMP3 positivity included Hodgkin's lymphomas (90%), neuroblastomas (88% positive), squamous cell cancers of various origins, e.g. of the lungs (81%), oral cavity (73%), esophagus (71%), larynx (67%), penis (59%), and skin (50%), as well as adenocarcinomas of the esophagus (74%), pancreas (62%), cervix (58%), stomach (50-55%), and lungs (51%). In contrast, IMP3 staining was only rarely

Table I. Summary of IMP3 immunohistochemical findings in 95 human types of cancer.

Tumor entity	On TMA (n)	Analyzable[a] (n)	IMP3 IHC results			
			Negative	Weak	Moderate	Strong
Skin tumors						
Skin, Merkel cell carcinoma	6	1	0%	100%	0%	0%
Skin, malignant schwannoma	14	11	36%	0%	18%	45%
Skin, squamous cell cancer	51	40	50%	10%	20%	20%
Skin, malignant melanoma	37	26	54%	0%	8%	38%
Pleomorphic sarcoma, NOS	25	19	74%	0%	0%	26%
Pilomatrixoma	48	24	75%	17%	4%	4%
Basalioma	67	39	79%	8%	8%	5%
Benign naevus	59	35	97%	3%	0%	0%
Skin, dermatofibrosarcoma protuberans	5	4	100%	0%	0%	0%
Lung tumors						
Lung, squamous cell cancer	59	37	19%	19%	14%	49%
Larynx, squamous cell cancer	57	39	33%	31%	13%	23%
Lung, large cell cancer	48	21	43%	0%	19%	38%
Lung, adenocarcinomas	71	39	49%	18%	5%	28%
Lung, malignant mesothelioma	28	6	50%	0%	0%	50%
Pharynx lymphoepithelial ca.	5	4	50%	0%	50%	0%
Lung, small cell lung cancer	15	2	50%	0%	50%	0%
Lung bronchioalveolar cancer	15	8	63%	0%	0%	38%
Gynecological tumors						
Cervix, adenosquamous ca.	3	3	33%	33%	0%	33%
Cervix, squamous cell carcinoma	63	58	62%	19%	5%	14%
Cervix, adenocarcinoma	48	24	42%	21%	4%	33%
Ovary, mucinous cancer	46	32	41%	16%	6%	38%
Ovary, serous cancer	63	53	55%	9%	13%	23%
Ovary, mullerian mixed type tumor	38	14	57%	0%	0%	43%
Ovary, endometrioid cancer	22	18	67%	11%	11%	11%
Uterus endometrial cancer	60	43	67%	14%	5%	14%
Vulva, squamous cell cancer	61	37	73%	16%	0%	11%
Granular cell tumor	8	5	80%	20%	0%	0%
Leiomyosarcoma	28	25	88%	0%	8%	4%
Ovary, Brenner	45	14	93%	0%	0%	7%
Mammary, apocrine type cancer	17	3	67%	0%	0%	33%
Mammary, medullary type cancer	64	42	76%	7%	10%	7%
Mammary, ductal type cancer	62	29	93%	3%	0%	3%
Mammary, lobular type cancer	65	23	100%	0%	0%	0%
Mammary, mucinous type cancer	61	22	100%	0%	0%	0%
Mammary, tubular type cancer	60	17	100%	0%	0%	0%
Mammary, cribriform type cancer	26	13	100%	0%	0%	0%
Mammary, phylloid type tumor	48	11	100%	0%	0%	0%
Uterus stromasarcoma	13	2	100%	0%	0%	0%
Uterus leiomyoma	27	20	100%	0%	0%	0%
Gastrointestinal tumors and head and neck tumors						
Esophagus, adenocarcinoma	60	31	26%	29%	29%	16%
Mouth, squamous cell cancer	54	33	27%	27%	9%	36%
Esophagus, squamous cell ca.	60	24	29%	33%	13%	25%
Pancreas, adenoca. ampulla	29	9	33%	33%	33%	0%
Pancreas, ductal adenocarcinoma	56	21	38%	29%	24%	10%
Intestinal cancer	22	7	43%	14%	14%	29%
Stomach, diffuse type cancer	56	11	45%	36%	9%	9%
Stomach, intestinal type cancer	62	18	50%	28%	0%	22%
Gallbladder cancer	30	18	50%	28%	11%	11%
Colon cancer	60	19	53%	16%	5%	26%

Table I. Continued. Summary of IMP3 immunohistochemical findings in 95 human types of cancer.

Tumor entity	On TMA (n)	Analyzable[a] (n)	IMP3 IHC results			
			Negative	Weak	Moderate	Strong
Gastrointestinal tumors and head and neck tumors						
Anal cancer	18	7	57%	29%	14%	0%
Colon, high grade adenoma	40	15	60%	33%	7%	0%
Hepatocellular carcinoma	55	23	61%	4%	4%	30%
Warthin tumor	57	39	79%	18%	3%	0%
Parotid gland, mucoepidermoid ca.	46	16	81%	0%	0%	19%
Colon, low grade adenoma	56	19	89%	5%	0%	5%
Basal cell adenoma	37	22	95%	0%	0%	5%
GIST	46	40	100%	0%	0%	0%
Parotid gland, pleomorphic adenoma	61	30	100%	0%	0%	0%
Pancreas, neuroendocrine ca.	20	5	100%	0%	0%	0%
Genitourinary tract tumors						
Testis, seminoma	92	69	0%	13%	16%	71%
Testis, non-seminoma	45	25	0%	4%	0%	96%
Teratoma	60	15	13%	7%	13%	67%
Oncocytoma	62	9	33%	22%	11%	33%
Penis, squamous cell cancer	46	22	41%	18%	14%	27%
Urinary bladder, T2-T4 tumors	60	56	61%	11%	4%	25%
Urinary bladder, Ta-Tumors	62	57	98%	2%	0%	0%
Kidney chromophobic	56	9	67%	11%	0%	22%
Kidney, papillary	31	8	75%	0%	0%	25%
Kidney, clear cell cancer	68	45	96%	2%	0%	2%
Prostate cancer	63	44	100%	0%	0%	0%
Neuroendocrine tumors						
Adrenal gland carcinoma	8	6	83%	0%	0%	17%
Adrenal gland adenoma	21	19	89%	5%	0%	5%
Thyroid, anaplastic cancer	3	1	0%	0%	0%	100%
Thyroid, medullary cancer	28	6	17%	0%	0%	83%
Thyroid, papillary cancer	54	15	87%	13%	0%	0%
Thyroid, follicular cancer	47	10	90%	0%	0%	10%
Thyroid, adenoma	65	22	91%	0%	0%	9%
Paraganglioma	36	29	93%	0%	0%	7%
Pheochromocytoma	64	48	100%	0%	0%	0%
Nervous system tumors						
Neuroblastoma	51	26	12%	4%	8%	77%
Astrocytoma	48	6	50%	17%	0%	33%
Oligodendriglioma	28	7	86%	0%	14%	0%
Medulloblastoma	4	2	100%	0%	0%	0%
Ependymoma	10	1	100%	0%	0%	0%
Soft-tissue tumors						
Chondrosarcoma	5	2	50%	0%	0%	50%
Angiosarcoma	7	5	60%	0%	20%	20%
Liposarcoma	16	14	86%	0%	7%	7%
Neurofibroma	60	21	100%	0%	0%	0%
Desmoid tumor	9	9	100%	0%	0%	0%
Synovia, giant cell carcinoma	40	9	100%	0%	0%	0%
Hemangiopericytoma	7	5	100%	0%	0%	0%
Lymphatic tumors						
Lymphoma, Hodgkin	43	10	10%	0%	90%	0%
Thymoma	57	43	98%	0%	0%	2%

[a]Analyzable refers to tumor cells containing spots on the tissue microarray.

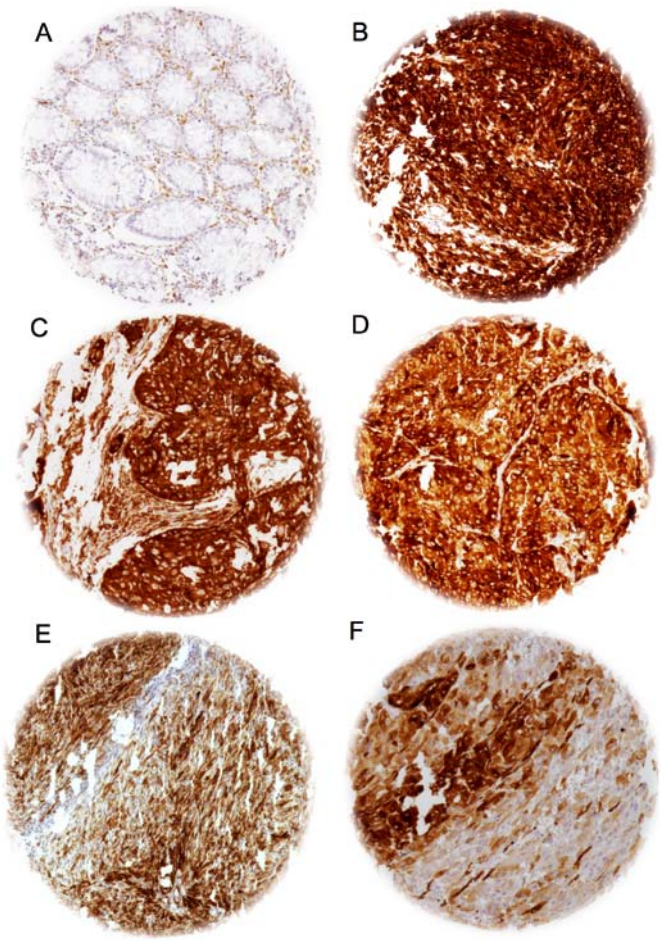

Figure 1. Examples of IMP3 staining in a case of (A) normal kidney tissue, (B) placenta, (C) seminoma, (D) non-seminoma (E) pancreatic cancer and (F) Hodgkin lymphoma.

found in breast cancers of no special type (7%) and clear cell renal cell cancers (4%). Examples of immunostaining of IMP3 positive and negative tissues are presented in Fig. 1.

The tumor types where IMP3 protein expression could never be detected included dermatofibrosarcomas of the skin (0 out of 4), subtypes of breast cancers (including lobular, mucinous, tubular cancers and phylloid tumors, 0 out of 11-23), leiomyomas (0 out of 20), stromal sarcomas (0 out of 2), GIST (0 out of 40), parotid adenomas (0 out of 30), neuroendocrine pancreatic cancers (0 out of 5), prostate cancers (0 out of 44), pheochromocytomas (0 out of 48), medulloblastomas (0 out of 2), ependymomas (0 out of 1), neurofibromas (0 out of 21), desmoid tumors (0 out of 9), synovial giant cell carcinomas (0 out of 9) and hemangiopericytomas (0 out of 5) and. However, the number of examined cases of several of these tumor types was low. These data therefore do not rule out that IMP3 expression can sometimes also occur in these tumor types.

Tumor type-specific prognostic TMAs. Expression data were available for 639 out of 697 (91.6%) urinary bladder cancers, 1204 out of 1711 (70.4%) colon cancers, 216 out of 343 (63%) adenocarcinomas of the esophagus, 170 out of 251 (67.7%) squamous cell carcinomas of the esophagus, 641 out of 763 (84%) lung cancers, 280 of 358 (78.2%) pancreas carcinomas, and 204 out of 230 (88,7%) stomach cancers on the prognostic TMAs.

IMP3 positivity was found in 21.9% of urinary bladder cancers (strong: 7.4%, moderate: 8.1%, weak: 6.4%), 63.4% of colon tumors (strong: 36%, moderate: 16.6%, weak: 10.7%), 74.1% of esophageal adenocarcinomas (strong: 50.5%, moderate: 11.1%, weak: 12.5%), 60.6% of esophageal squamous cell cancers (strong: 41.8%, moderate: 8.2%, weak: 10.6%), 63.2% of lung cancers (strong: 47.1%, moderate: 11.4%, weak: 4.7%), 48.9% of pancreatic cancers (strong: 8.2%, moderate: 10.7%, weak: 30%), and 44.9% of stomach cancers (strong: 24%, moderate: 10.8%, weak: 20.1%).

Significant associations were found between IMP3 and advanced stage and grade in urinary bladder cancers ($P<0.0001$ each, Fig. 2A), in high grade and metastatic phenotype in esophageal adenocarcinomas ($P\leq0.0025$, Fig. 2B), in a squamous cell phenotype in lung cancers ($P<0.0001$, Fig. 2C), and in shortened survival in adenocarcinomas of the lungs ($P=0.0175$, Fig. 2D), in stomach ($P=0.0032$, Fig. 2E) and in pancreatic cancers ($P=0.0304$, Fig. 2F). No significant associations between IMP3 expression levels and patient prognosis were observed in urinary bladder cancers, squamous cell lung cancers and squamous cell esophageal cancers.

Discussion

The results of our study provide a comprehensive catalogue of IMP3 expression across a large variety of human types of cancer and subtypes. Our findings demonstrated that high levels of IMP3 protein expression were found in the vast majority of the analyzed types of cancer and underscored its considerable general importance in tumor biology. The novelty of this study is that staining was performed with a single antibody, in 1 laboratory on a large panel of different tumor entities on a single microarray.

The frequency of IMP3 expression in individual types of cancer is well in the range of that reported from previous research (Fig. 3), which corroborates the validity of our results. For example, virtually all analyzed testicular cancers; teratomas, Hodgkin's lymphomas and Merkel cell cancers of the skin were IMP3-positive under the experimental conditions selected for this study, which fits well with the 85-99% positivity reported in the literature (4,34-36). In contrast, and in concordance with previous research, tumor types largely lacking detectable IMP3 expression included breast cancers of no special type, gastrointestinal stromal tumors, desmoid tumors, benign naevi or leiomyomas, which all have been described as predominantly IMP3-negative before (21,37,38). More variable findings with respect to published data were made in tumor types with intermediate IMP3-positivity, such as hepatocellular carcinomas (53-68% in the literature; 40% in our study) or colon cancers (15-65% in the literature; 47% in our study). It is obvious, that different antibodies and immunohistochemistry protocols, scoring criteria and the comparatively small number of samples (usually <50 per tumor category in our study) account for these differences.

There were 14 tumor types that were newly identified as having occasional IMP3 protein overexpression in this study. These included many important types of cancer such as squamous cell cancers of the vagina and vulva, medullary breast

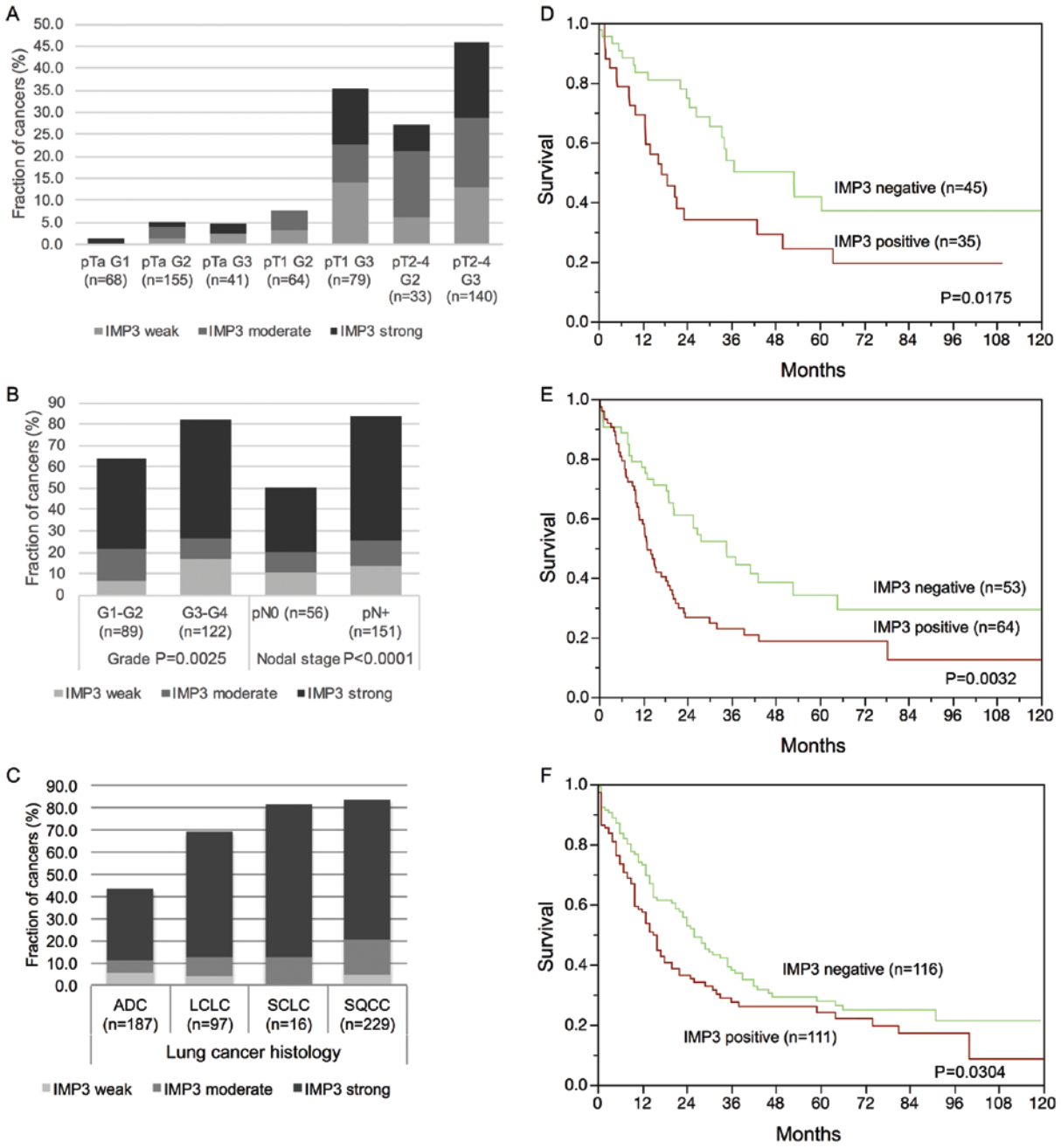

Figure 2. Associations between IMP3 staining results, tumor phenotypes and patient prognosis in different types of cancer. Association between IMP3 expression and (A) advanced tumor stage and high grade in urinary bladder cancer, (B) high grade and metastatic phenotype in esophageal adenocarcinomas, (C) squamous cell phenotype in lung cancer, (D) shortened survival in lung adenocarcinoma, (E) shortened survival in stomach cancer, and (F) shortened survival in pancreatic cancer.

and thyroid cancers, pharyngeal carcinomas, various sarcomas (including chondro-, angio- and liposarcomas), oncocytomas, as well as some types of neuronal cancers (e.g. paragangliomas and oligodendrigliomas). These findings further add to the growing list of IMP3-expressing types of cancer and emphasize the general role of IMP3 as a marker for malignant growth of tumors arising from epithelial, mesenchymal and neuronal tissues.

Since IMP3 is often referred to as a marker for distinguishing between benign and malignant lesions (39-43), it was of interest to also find at least occasional expression in a few individual cells of glandular epithelium, lymphatic tissues and placenta, which was in accordance to earlier studies demonstrating that IMP3 was ubiquitously expressed in early developmental stages of human tissues but also in adult placenta (44,45). In addition, we also found IMP3 expression in benign lesions such as Warthin's tumors, schwannomas, colon adenomas and basal cell adenomas. Such findings are often made in studies including multiple types of tumors and demonstrate that the specificity of molecular markers are often overestimated in initial studies including only a limited number of different samples. For example, IMP3 has been suggested to aid in the diagnosis of melanoma, leiomyosarcoma, HCC, papillary thyroid carcinoma, and follicular thyroid carcinomas, but it is not a sensitive marker for these tumors as lack of IMP3 expression in these tumors cannot exclude a malignant phenotype (reviewed in ref. 1).

Figure 3. Summary of literature findings on the prevalence of IMP3 expression in human cancers: Red circles indicate the results of the current study. Findings from the literature (4-6,8,9,12-27,34-37,42,46,48,62-95) sequence of citations equals the sequence of the tumor types from top to bottom) are marked by gray circles.

In addition to analyzing the molecular epidemiology of IMP3 expression, we took advantage of various pre-existing tumor type-specific prognostic TMAs for evaluation of the clinical significance of IMP3 expression in several tumor types. Urinary bladder, colon, esophageal, lung, pancreatic and stomach cancer were selected for follow-up studies because IMP3 was frequently positive in these tumor types and large TMAs were available. Significant associations between IMP3 expression and tumor phenotype as well as patient prognosis in our study further added to the knowledge on the clinical impact of IMP3 in these tumor types, which have been only studied in comparatively small cohorts of usually less than 100 cancer samples thus far. For example, the strong associations between IMP3 expression and advanced stage and grade in urinary bladder cancers and esophageal adenocarcinomas, or a squamous cell phenotype in lung cancers are in line with previous work on 76-384 cancers of the urinary bladder (23,24), 147 esophageal cancers (46) and 89-224 lung cancers (reviewed in refs. 47,48). Furthermore, the adverse prognostic impact of IMP3 expression in lung adenocarcinomas, pancreatic cancers and stomach cancers in our study were supported by previous studies on 40-190 patients in

these types of cancer (10,11,48-50). In addition a recent meta-analysis revealed a hazard ratio of 2.08 for decreased survival in solid tumors expressing high levels of IMP3 (51).

Our analysis provides a comprehensive overview on IMP3 protein expression in neoplastic human tissues. Tissue micro-arrays are an ideal tool to massively accelerate characterization of novel biomarkers. The use of TMAs to jointly screen many different tumor types for molecular alterations of interest is an obvious application of this technique. In earlier studies we had used multi-tumor TMAs for the evaluation of cyclin E (52), calretinin (53), KIT (54), ERG (55) or copy number changes of 17q23 (56). Others have used comparable TMAs to evaluate SPANX-B (57), SIL (58), NOX1 (59), COX2, MMP2, or MMP9 (60).

It is a distinct advantage of the TMA technique that all tissues are analyzed under maximally standardized conditions. While 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 immunostaining. For example, TMAs overcome the issue of slide ageing and decreased immunoreactivity (61) that can become a serious problem in conventional large studies where tissue sections are often stored over a longer period of time prior to analysis, because all tissue samples in a TMA can be easily sectioned and stained within one day.

In summary, the results of our study show that IMP3 expression is a common feature of most human solid cancer types, but may also be observed in benign lesions. Strong IMP3 expression is often linked to adverse tumor features.

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