BIOMARKERS, GENOMICS, PROTEOMICS, AND GENE REGULATION

Glypican 3 Overexpression across a Broad Spectrum of Tumor Types Discovered with Functional Genomic mRNA Profiling of a Large Cancer Database

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Glypican 3 (GPC3), a membrane-bound heparan sulfate proteoglycan, is overexpressed in approximately 70% to 80% of hepatocellular carcinomas, but is not expressed commonly in healthy tissues. This raised interest in GPC3 as a drug target and several GPC3-targeting drugs are in clinical development. We therefore predicted GPC3 protein overexpression across tumors and validated these predictions. Functional genomic mRNA profiling was applied to the expression profiles of 18,055 patient-derived tumor samples to predict GPC3 overexpression at the protein level in 60 tumor types and subtypes using healthy tissues as reference. For validation, predictions were compared with immunohistochemical (IHC) staining of a breast cancer tissue microarray and literature data reporting IHC GPC3 overexpression in various solid, hematologic, and pediatric tumors. The percentage of samples with predicted GPC3 overexpression was 77% for hepatocellular carcinomas ($n = 364$), 45% for squamous cell lung cancers ($n = 405$), and 19% for head and neck squamous cell cancers ($n = 344$). Breast cancer tissue microarray analysis showed GPC3 expression ranged from 12% to 17% in subgroups based on estrogen receptor and human epidermal growth factor receptor 2 status. In 28 of 34 tumor types for which functional genomic mRNA data could be compared with IHC there was a relative difference of $\leq 10\%$. This study provides a data-driven prioritization of tumor types and subtypes for future research with GPC3-targeting therapies. (*Am J Pathol* 2018, 188: 1973–1981; <https://doi.org/10.1016/j.ajpath.2018.05.014>)

In personalized medicine, identification of targetable tumor-specific or tumor-associated characteristics to increase therapeutic possibilities in cancer patients is of great value. Although many treatment protocols have been enhanced with novel drugs including molecularly targeted agents that inhibit specific oncogenic driver pathways, not all patients benefit because driver targets are not available for all tumor types. Interestingly, antigen targets for novel therapeutic approaches such as bispecific antibodies, antibody-drug conjugates, antibodies, or antibody fragments fused with a toxin, radioimmunoconjugates, and chimeric antigen receptors, do not have to be drivers of tumor growth because their task is to serve as an anchor to bind the compounds. This clearly increases the total number of available antigen targets in cancer.

In this context, glypican 3 (GPC3), a membrane-bound heparan sulfate proteoglycan, is an interesting antigen target. During embryogenesis, GPC3 is expressed abundantly in multiple tissues.¹ After birth, GPC3 expression rarely is observed in healthy tissues, although overexpression is seen in regenerating tissues.² For example, GPC3 overexpression is present in up to 83% of chronic nontumor cirrhotic livers; however, expression in healthy liver tissue and benign liver lesions is rare.^{3–8} In addition, GPC3

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Figure 1 Glypican 3 (GPC3) overexpression rates in solid tumor types as determined with functional genomic mRNA (FGmRNA) profiling or immunohistochemical (IHC) analyses derived from our breast cancer tissue microarray (TMA) or literature data. The x axis shows the percentage of samples with overexpression of GPC3. Tumor types including relevant subgroups are shown on the y axis. CNS, central nervous system; ER, estrogen receptor; HER2, human epidermal growth factor receptor 2; TNBC, triple negative breast cancer.

overexpression is found in several tumors, most notably in approximately 70% to 80% of hepatocellular carcinomas (HCCs), but also in yolk sac tumors, gastric carcinoma, colorectal carcinoma, non-small cell lung cancer, and thyroid cancer.^{9–17}

GPC3 has specific characteristics that make it a potentially attractive drug target: protein expression is absent or at low levels in healthy adult tissues, it is located at the cell surface, and it is overexpressed by several tumor types. In

Figure 2 Glypican 3 (GPC3) overexpression rates in solid tumor types as determined with functional genomic mRNA (FGmRNA) profiling or immunohistochemical (IHC) analyses in literature. The x axis shows the percentage of samples with overexpression of GPC3. Tumor types including relevant subgroups are shown on the y axis. HNSCC, head and neck squamous cell cancer.

Figure 3 Glypican 3 (GPC3) overexpression rates in hematologic and pediatric tumors as determined with functional genomic mRNA (FGmRNA) profiling or immunohistochemical (IHC) analyses in literature. The x axis presents the percentage of samples with overexpression of GPC3. Tumor types are shown on the y axis. CNS, central nervous system.

this respect, it is critical to have good insight into its overexpression across several tumor types.

Immunohistochemical (IHC) analysis enables investigation of protein overexpression of GPC3 in various tumor types and subtypes. However, gaining insight into a broad range of tumors using IHC screening for the presence of this druggable target is time consuming and demands many resources. Functional genomic mRNA profiling (FGmRNA profiling) therefore was used to predict overexpression of GPC3 at the protein level.¹⁸ An advantage of this method is that it can correct a gene expression profile of an individual tumor for physiological and experimental factors that may not be relevant for the observed tumor phenotype.

In this study, FGmRNA profiling was applied to a large database containing a broad spectrum of tumor types and subtypes to predict GPC3 protein overexpression for each tumor type/subtype, using healthy tissue samples as reference. The predictions from FGmRNA profiling then were validated by comparing them with IHC staining of a breast cancer tissue microarray (TMA), derived from tumors of an independent set of patients. In addition, predicted GPC3 overexpression was compared with historical GPC3 protein overexpression IHC data derived from the literature.

Materials and Methods

Acquisition of Expression Data

Publicly available microarray expression data were extracted (Gene Expression Omnibus, <https://www.ncbi.nlm.nih.gov/geo>).¹⁹ Gene Expression Omnibus accession numbers are provided in [Supplemental Table S1](#). The analysis was restricted to the Affymetrix HG-U133 Plus 2.0 (GPL570) platform (Affymetrix, Santa Clara, CA). For each sample, metadata including patient information and experimental conditions were collected in the simple omnibus format in text file format. Relevant samples were selected using a two-step approach: automatic filtering on relevant keywords followed by manual curation. Samples were retained when raw data (CEL files) were available and when the samples were representative tumor tissue samples of patients or healthy tissue samples. Preprocessing and aggregation of raw data were performed according to the robust multiarray average algorithm with RMAExpress (version 1.1.0) using the latest CDF file from Affymetrix.²⁰ Quality control of the resulting expression data were performed as previously described.^{18,21,22} A message-digest algorithm 5 hash for each

Table 1 Immunohistochemical Analysis of GPC3 Overexpression in a Breast Cancer Tissue Microarray Containing 391 Tumor Samples

Tumor-receptor status	GPC3-positive tumor samples, n/total (%)	Tumor samples showing cytoplasmic staining, n*	Tumor samples showing nuclear staining, n*
ER positive	28/211 (13)	7 IHC1 ⁺ , 6 IHC2 ⁺	6 IHC1 ⁺ , 9 IHC2 ⁺
HER2 positive [†]	5/30 (17)	2 IHC1 ⁺ , 1 IHC2 ⁺	3 IHC1 ⁺
ER positive/HER2 positive	10/86 (12)	2 IHC1 ⁺ , 2 IHC2 ⁺ , 1 IHC3 ⁺	5 IHC1 ⁺
TNBC	7/56 (13)	1 IHC1 ⁺ , 1 IHC2 ⁺ , 3 IHC3 ⁺	2 IHC1 ⁺

*Subdivided into staining intensity.

[†]One core showed both cytoplasmic (IHC2⁺) and nuclear (IHC2⁺) staining and therefore is represented twice in the table.

ER, estrogen receptor; GPC3, glypican 3; HER2, human epidermal growth factor receptor 2; IHC, immunohistochemically; TNBC, triple negative breast cancer.

Figure 4 Representative examples of immunohistochemical glypican 3 (GPC3) staining results. Positive GPC3 staining is shown in brown. **A:** Strong nuclear staining of GPC3 in breast cancer. **B:** Weak nuclear staining of GPC3 in breast cancer. **C:** Strong GPC3 cytoplasm staining in breast cancer. **D:** Weak GPC3 cytoplasm staining in breast cancer. **E:** Strong GPC3 staining in placenta tissue (positive control). **F:** Negative staining of GPC3 in kidney tissue (negative control). Original magnification, $\times 40$.

individual CEL file was used to identify and remove duplicate CEL files. For the breast cancer cohort, receptor status was collected or inferred as described previously.^{23–25}

Predicting Protein Overexpression of GPC3 with FGmRNA Profiling

The FGmRNA profiling method is described in detail by Fehrmann et al.¹⁸ In short, 77,840 expression profiles of publicly available samples were analyzed with principal component analysis and it was found that a limited number of transcriptional components captured the major regulators of the mRNA transcriptome. Subsequently, a subset of transcriptional components that described nongenetic regulatory factors were identified. These nongenetic transcriptional components were used as covariates to correct microarray expression data and it was observed that the residual expression signal (ie, the FGmRNA profile) captured the downstream consequences of genomic alterations on gene expression levels.

The percentage of samples per tumor type, including relevant subgroups, then was predicted based on histotype (eg, adenocarcinoma) or receptor status (eg, breast cancer) with an increased FGmRNA signal for GPC3, which was used as a proxy for protein overexpression. The threshold was defined in the set of FGmRNA profiles of healthy tissues by calculating the 97.5th percentile for the FGmRNA signal of GPC3. For each tumor sample, GPC3 was marked as overexpressed when the FGmRNA signal was above the 97.5th percentile threshold as defined in the healthy tissue samples. Because the Affymetrix HG-U133 Plus 2.0

platform contains two probes representing GPC3, the highest percentage of samples with an increased FGmRNA signal was systematically reported.

Immunohistochemical GPC3 Staining of Breast Cancer TMA

Seven breast cancer TMAs containing residual tumor samples of patients treated for primary breast cancer in the University Medical Center Groningen between 1996 and 2005 were stained for GPC3. TMA construction and validation for breast cancer was described previously.^{26,27} In brief, TMAs were constructed as follows: the most representative tumor area was marked on a hematoxylin and eosin–stained section. By using the hematoxylin and eosin–stained section for orientation, three 0.6-mm cores were taken from the selected area in the donor blocks and mounted on a recipient block, using a manual TMA device (Beecher Instruments, Silver Springs, MD). After this, 3- μ m sections were cut from these TMA blocks using a standard microtome.

Tumor samples were stained using an anti-GPC3 antibody (clone 1G12, dilution 1:100; BioMosaics, Burlington, VT) on an automated Benchmark Ultra stainer (Ventana Medical Systems, Inc., Tucson, AZ). Normal placenta was used as a positive control and normal kidney tissue was used as a negative control.

Two authors (K.L.M. and D.J.A.d.G.) independently scored three cores of each tumor sample for staining intensity. Immunostains were excluded from IHC analysis if they were unrepresentative or unscorable owing to technical

issues (eg, incomplete tissue transfer to the microscopic slide). The staining intensity was scored semiquantitatively as follows: 0, negative; 1+, weak; 2+, moderate; and 3+, strong; as described by Hirabayashi et al.⁶ A tumor sample was considered positive when weak, moderate, or strong GPC3 staining was seen in at least 5% of tumor cells within at least one core. When staining was present in >1 core of one tumor sample the highest staining intensity consistently was reported. Different staining patterns (cytoplasmic or nuclear) were described. In case of a discrepancy between the two observers, a breast pathologist (B.v.d.V.) independently scored the tumor sample during a consensus meeting and a final verdict was reached.

Literature Search

To collect IHC data for GPC3 protein overexpression in cancer, PubMed was searched in April 2017 for relevant articles published in English. The following search terms were used in different combinations and spelling variants: "immunohistochemistry," "expression," "glypican 3," "GPC3," "cancer," "tumor." The retrieved articles were completely screened for the presence of IHC staining of patient tumor tissue. Case reports and reviews were excluded. Subsequently, the number of tumor samples analyzed and the percentages of tumor samples marked as GPC3 positive were recorded per tumor type and per article. GPC3 positivity was defined as being present when it was determined as positive in the original article. In addition, ClinicalTrials.gov was searched for ongoing studies with GPC3-directed therapies on June 26, 2017. The search terms "GPC3" or "glypican" were used.

Results

Predicted Protein Overexpression of GPC3 by FGmRNA Profiling

A total of 18,055 samples representing 60 tumor types, including relevant subgroups, and 3520 samples representing 22 healthy tissue types were identified. The median number of tumor samples analyzed per tumor type or subtype was 88 (interquartile range, 33 to 343), ranging from 7 in Burkitt lymphoma to 2710 in colorectal cancer.

A predicted GPC3 overexpression rate in 77% of samples was observed for HCC, 45% for squamous cell lung cancer, 19% for head and neck squamous cell cancer, and 18% for squamous cell esophageal cancer. In lung cancer and esophageal cancer, the squamous cell histologic subtype showed higher predicted GPC3 overexpression compared with adenocarcinomas.

In breast cancer, the predicted GPC3 overexpression was receptor status-dependent, with 13% for estrogen receptor positive, 7% for human epidermal growth factor receptor 2

positive, 14% for estrogen receptor positive/human epidermal growth factor receptor 2 positive, and 8% for triple-negative breast cancers. In total, 22 of 60 tumor types and subtypes studied showed predicted overexpression in $\geq 10\%$ of samples (Figures 1, 2, and 3). Predicted GPC3 overexpression in at least 1% of samples was found in 51 of 60 tumor types and subtypes, including 8% for prostate cancer and 7% for colorectal cancer. Predicted GPC3 protein overexpression for all tested solid, hematologic, and pediatric tumors is shown in Figures 1, 2, and 3, additional information is provided in Supplemental Table S2.

IHC Determined Protein Overexpression of GPC3 in a Breast Cancer TMA

A total of 391 tumor samples, with an average of 2.74 assessable cores per tumor, were studied. GPC3 overexpression ranged from 12% to 17% in subgroups based on estrogen receptor and human epidermal growth factor receptor 2 status (Table 1). Both GPC3 cytoplasm and nuclear staining patterns of tumor cells were present in various intensities. Figure 4 shows representative staining patterns of GPC3 in breast cancer. Thirty tumor samples were unrepresentative or unscorable and therefore were excluded from analyses.

Literature-Based GPC3 Protein Expression

In total, 166 studies were identified that used IHC to determine GPC3 protein overexpression in 107 different tumor types and subtypes in 20,653 tumor samples. The number of samples analyzed per tumor type and subtype varied between 1 for Hürthle cell thyroid cancer to 8446 for HCCs, with a median of 49 (interquartile range, 18 to 147). In total, 19 different antibodies were used, of which the 1G12 monoclonal antibody from BioMosaics (1G12; Cell Marque, Rocklin, CA; and 1G12; Santa Cruz Biotechnology, Dallas, TX) was applied most frequently. Seventy different GPC3-positivity scoring systems were used.

In Table 2, GPC3 protein overexpression rates are shown for tumor types and subtypes for which data from two or more articles were available. Data concerning GPC3 overexpression in additional tumor types and subtypes are shown in Supplemental Table S3.

GPC3 Overexpression Rates Predicted by FGmRNA Profiling Compared with IHC Data for a Breast Cancer TMA or Historical IHC Data in Various Tumor Types as Reported in Included Articles

For 34 tumor types and subtypes, both FGmRNA profiling and IHC protein data were available (Figures 1, 2, and 3). For 19 of these tumor types and subtypes, the GPC3 protein expression predicted by FGmRNA profiling was higher than

Table 2 Published Results Regarding Immunohistochemically Measured GPC3 Overexpression in Tumors

Tumor types and subtypes	Articles, <i>n</i>	Patients reported, <i>n</i>	Median GPC3 positivity across all articles, %	IQR
Breast cancer				
Ductal	2	147	5	NA
Lobular	2	94	12	NA
CNS tumors				
Astrocytoma	3	78	0	NA
Atypical teratoid rhabdoid tumor	3	34	77	NA
Ependymoma	2	24	4	NA
Glioblastoma	2	62	1	NA
Meningioma	2	59	3	NA
Neuroblastoma	2	171	2	NA
Oligodendroglioma	2	37	2	NA
Thyroid cancer				
Anaplastic	2	22	0	NA
Follicular	3	60	67	NA
Medullary	2	15	40	NA
Papillary	6	239	25	0–73
Gastrointestinal cancer				
Anal SCC	3	25	20	NA
Cholangiocarcinoma	12	173	0	0–8
Cholangiocarcinoma, intrahepatic	11	417	2	0–6
Colorectal adenocarcinoma*	7	348	2	0–59
Esophageal adenocarcinoma	2	38	16	NA
Esophageal SCC	3	63	27	NA
Gallbladder cancer	3	160	7	NA
Gastric adenocarcinoma	12	1713	14	1–27
Gastric cancer AFP producing	3	53	96	NA
HCC	98	8446	76	61–83
HCC, cholangiocarcinoma combined	6	170	65	38–88
HCC, fibrolamellar	9	135	20	17–60
Hepatoblastoma	6	150	100	90–100
Pancreatic adenocarcinoma	3	89	0	NA
Pancreatic cancer	4	75	42	6–86
Small-bowel cancer	2	14	29	NA
Gynecological cancer				
Cervical SCC	2	48	19	NA
Endometrioid	2	101	6	NA
Endometrioid adenocarcinoma	2	49	4	NA
Endometrioid serous carcinoma	2	42	20	NA
Ovarian clear cell cancer	6	261	41	26–49
Ovarian endometrioid cancer	4	152	8	5–9
Ovarian serous cancer	5	523	11	1–15
Lung cancer				
Adenocarcinoma	9	476	8	4–15
Large-cell carcinoma	2	59	27	NA
Mesothelioma	2	35	4	NA
SCC	8	362	52	42–63
Small-cell carcinoma	2	59	13	NA
Skin cancer				
Basal cell carcinoma	2	59	7	NA
Melanoma	5	233	0	0–55
Sarcoma				
Epithelioid sarcoma	2	58	1	NA
Ewing sarcoma	2	15	0	NA
Fibrosarcoma	2	41	9	NA
Leiomyosarcoma	3	237	3	NA
Rhabdomyosarcoma	5	362	20	22–33

(table continues)

Table 2 (continued)

Tumor types and subtypes	Articles, <i>n</i>	Patients reported, <i>n</i>	Median GPC3 positivity across all articles, %	IQR
Synovial sarcoma	2	76	3	NA
Undifferentiated	3	140	50	NA
Urogenital cancer				
Bladder cancer	4	97	15	10–29
Prostate cancer	3	251	3	NA
Urothelial cancer	6	562	3	0–18
Renal cancer				
Chromophobe	4	93	14	2–65
Clear cell	4	654	1	0–4
Oncocytoma	3	40	11	NA
Papillary	4	149	5	1–21
Wilms tumor	3	87	38	NA
Germ cell tumors				
Dysgerminomas	2	29	10	NA
Extragenital YST	6	125	100	85–100
Germ cell tumors NOS [†]	3	54	44	NA
Nondysgerminomas	9	153	100	61–100
Nonseminomas	11	430	52	43–100
Seminomas	7	243	0	0–8
Other				
Malignant rhabdoid tumor	3	34	12	NA
NET/NEC	5	321	0	0–2
Salivary gland tumor	2	71	3	NA

*One article did not specify the histologic subtype of colorectal patients.

[†]Testicular/ovarian origin not clearly specified.

AFP, α -fetoprotein producing; CNS, central nervous system; GPC3, glypican 3; HCC, hepatocellular carcinoma; IQR, interquartile range; NA, not applicable; NEC, neuroendocrine carcinoma; NET, neuroendocrine tumor; NOS, not otherwise specified; SCC, squamous cell cancer; YST, yolk sac tumor.

indicated by IHC data. For 16 of these, the relative difference was less than 10%. The largest discrepancy was seen for leiomyosarcoma. For this tumor, a GPC3 protein overexpression of 35% was predicted by FGmRNA profiling ($n = 60$), compared with 3% indicated by IHC analysis in three studies ($n = 237$). For 13 tumor types and subtypes a higher rate of GPC3 expression in tumors was reported with IHC compared with FGmRNA profiling. For 10 of this group, the relative difference was $\leq 10\%$. In liposarcoma, GPC3 protein overexpression was present in 52% of the 29 cases with IHC analysis in one study compared with 11% with FGmRNA profiling ($n = 76$). For estrogen receptor–positive breast cancer (13%) and neuroblastoma (2%), FGmRNA profiling and IHC showed the exact same results.

Discussion

This study shows that FGmRNA profiling can be used as a screening tool to predict GPC3 overexpression across 60 tumor types and subtypes as validated by comparison with IHC staining of a breast cancer TMA and literature data reporting IHC GPC3 overexpression in tumors. In HCC, squamous cell lung cancer and head and neck squamous cell cancer the percentages of samples with predicted GPC3

overexpression were 77%, 45%, and 19%, respectively, and these tumor types and subtypes are therefore of interest for GPC3-directed therapies.

In high-incidence tumor types such as colorectal cancer, breast cancer, and prostate cancer, less than 15% of samples overexpress GPC3. Although GPC3 expression was $<15\%$ in 78% of the tumor types and subtypes studied, this can be relevant to the increasing use of personalized treatment strategies. This is exemplified by the 8% of triple negative breast cancers that had GPC3 overexpression. For this highly aggressive breast cancer subtype, targeted treatment options are lacking.²⁸ Recently, the US Food and Drug Administration approved for the first time a cancer treatment based on a tumor biomarker: pembrolizumab for treatment of microsatellite instability-high or mismatch-repair–deficient advanced solid tumors independent of the tumor's original location. This approval might pave the way for registration of additional compounds in the near future to treat tumors with specific features, such as GPC3 overexpression, independent of their origin.

There are some pitfalls concerning FGmRNA profiling, which was used as a screening tool to assess GPC3 overexpression across a very large set of samples representing many tumor types and subtypes. For example, mRNA might not always be translated to protein or the protein might not end up on the cell membrane and therefore mRNA data

must be interpreted with some caution.²⁹ In addition, FGmRNA profiling does not inform about target heterogeneity and it cannot distinguish between tumor cells and nontumor cells as a source of target overexpression. However, FGmRNA profiling does offer an educated guess to answer questions concerning antigen target overexpression across tumors in a more efficient manner than large-scale IHC analyses, which is used most often in the clinic to assess protein presence. Subsequent IHC validation might be warranted.

To validate predicted GPC3 overexpression at the protein level, the predictions were compared with our own IHC analysis in breast cancer patients or historical IHC data from the literature. In breast cancer, comparable results were shown for GPC3 overexpression in all subgroups, for other tumor types and subtypes corresponding results were found in the majority of tumor types and subtypes. However, there were some limitations to this comparison with historical IHC data in literature. Major heterogeneity in staining antibodies, scoring methods, and cut-off boundaries for GPC3 positivity in the 166 included studies hampered direct comparison. It has been clearly illustrated that a lack of standardized protocols has had a strong impact on IHC results.³⁰

In addition to IHC, other techniques such as mass spectrometry or Western blot analysis also can be applied to study protein abundance in tissues. However, all of these techniques require the availability of tumor tissue samples and therefore come with limitations including invasiveness, procedural risks, and accessibility of tumor to biopsy. In addition, only static information is provided and heterogeneity is not taken into account. Alternatively, molecular imaging, defined as the visualization, characterization, and measurement of biological processes at molecular and cellular levels, can be used to study protein expression.³¹ This tool provides whole-body information regarding (heterogeneity in) protein expression in a noninvasive manner and increasingly is being implemented in drug development and patient care.³² FGmRNA profiling can be used as a tool to select suitable targets for molecular imaging, as has been shown by Koller et al³³ for pancreatic ductal adenocarcinomas.

These data show the percentage of patients per tumor type and subtype who might benefit from GPC3-directed therapies. GPC3-targeting monoclonal antibodies, bispecific T-cell—redirecting antibodies, peptide vaccinations, dendritic cell vaccinations, and chimeric antigen receptors have been engineered and mainly are being explored in early clinical trials involving HCC patients.^{34–39} Potentially interesting results have been seen with peptide vaccinations, provoking partial responses in a subset of patients with advanced HCC or refractory ovarian clear cell carcinoma.^{34,40} Dendritic cell vaccine therapy increased the 5-year recurrence-free survival rate in a few HCC patients who received dendritic cell vaccine therapy as compared with 31 historical control patients.³⁶ The role of GPC3-directed bispecific

T-cell—redirecting antibodies and chimeric antigen receptors currently are being explored in HCC (ClinicalTrials.gov; identifiers NCT02905188, NCT02723942, NCT02715362, NCT02395250, NCT02959151, NCT03130712, NCT03084380, and NCT03146234), squamous cell lung carcinoma (identifier NCT02876978), and (pediatric) solid tumors (NCT02748837 and NCT02932956).

In conclusion, the present study provides a data-driven prioritization of tumor types for future research with GPC3-targeting therapies.

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We thank the other studies using IHC to determine GPC3 protein overexpression that could not be included because of spatial limitations.

Supplemental Data

Supplemental material for this article can be found at <https://doi.org/10.1016/j.ajpath.2018.05.014>.

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