

Patterns of *TPD52* overexpression in multiple human solid tumor types analyzed by quantitative PCR

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Abstract. Tumor protein D52 (TPD52) is located at chromosome 8q21, a region that is frequently gained or amplified in multiple human cancer types. TPD52 has been suggested as a potential target for new anticancer therapies. In order to analyze TPD52 expression in the most prevalent human cancer types, we employed quantitative PCR to measure TPD52 mRNA levels in formalin-fixed tissue samples from more than 900 cancer tissues obtained from 29 different human cancer types. TPD52 was expressed at varying levels in all tested normal tissues, including skin, lymph node, lung, oral mucosa, breast, endometrium, ovary, vulva, myometrium, liver, pancreas, stomach, kidney, prostate, testis, urinary bladder, thyroid gland, brain, muscle and fat tissue. *TPD52* was upregulated in 18/29 (62%) tested cancer types. Strongest expression was found in non-seminoma (56-fold overexpression compared to corresponding normal tissue), seminoma (42-fold), ductal (28-fold) and lobular breast cancer (14-fold). In these tumor types, TPD52 upregulation was found in the vast majority (>80%) of tested samples. Downregulation was found in 11 (38%) tumor types, most strongly in papillary renal cell cancer (-8-fold), leiomyosarcoma (-6-fold), clear cell renal cell cancer (-5-fold), liposarcoma (-5-fold) and lung cancer (-4-fold). These results demonstrate that TPD52 is frequently and strongly upregulated in many human cancer types, which may represent candidate tumor types for potential anti-TPD52 therapies.

Introduction

Copy number increase involving chromosome 8q belong to the most frequent aberrations in human solid cancers, including

for example tumors of the breast, ovary, endometrium, lung, colon, head and neck, urinary bladder, kidney and prostate (1,2). Whereas gains often affect large portions of 8q or even the entire q-arm, high-level amplifications are typically focused on the chromosomal bands 8q21 (3-5) and 8q24 (6,7), thus highlighting the loci of putative oncogenes, including MYC at 8q24. Several candidate oncogenes have been suggested to reside inside the 8q21 region, including tumor protein D52 (TPD52) (3,8-13). *TPD52* has been suggested to play a role for vesicle trafficking and Ca²⁺ dependent exocytotic secretion, and has been shown to facilitate cytokinesis in rapidly proliferating cells (14,15).

In line with an oncogenic role, TPD52 overexpression has been described from a multitude of cancer types, including breast (9), lung prostate (2), ovarian (8), pancreatic cancer (16), multiple myeloma (17,18), Burkitt's lymphoma (19), melanoma (20) and testicular germ cell tumors (21), and has been linked to poor prognosis in breast, medulloblastoma, lung and prostate cancer patients (22). Moreover, cell line experiments and *in vivo* analysis in mice support the implication of *TPD52* in tumorigenesis and progression to metastasis (23,24). Accordingly, *TPD52* has been suggested as a promising target for antitumor therapies in breast (25) and prostate cancer (24), and it seems likely that also other tumor types showing *TPD52* overexpression could profit from a *TPD52* specific therapy.

A systematic analysis of TPD52 expression in human cancers in order to identify tumor types that might benefit from potential anti-TPD52 therapies is lacking. In this study, we have employed quantitative real-time PCR in more than 900 tumor samples to compare the prevalence and expression levels of *TPD52* across 29 important human cancer types and corresponding normal tissues.

Materials and methods

Tissue specimens. Formalin-fixed, paraffin embedded tissues were selected from the archive of the Institute of Pathology, University Medical Center Hamburg-Eppendorf (Hamburg, Germany). A total of 999 cancer samples and 40 normal tissue samples were included into the study. A detailed list of all samples is given in Table I. One pathologist reviewed all hematoxylin and eosin stained sections of all tissues and selected one block per tumor for RNA isolation. For tumor

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Table I. Tissue samples for *TPD52* expression analysis.

Tissue type	Organ	n	Organ	n
Normal	Skin	2	Pancreas	1
	Lymph node	2	Stomach	2
	Lung	2	Kidney	2
	Oral cavity	2	Prostate	2
	Breast	1	Testis	3
	Endometrium	2	Bladder	2
	Ovar	2	Thyroid gland	2
	Vulva	2	Brain	2
	Myometrium	2	Skeletal muscle	2
	Liver	3	Adipose tissue	2
Cancer	Malignant melanoma	11	Liver cancer	50
	Larynx carcinoma	39	Pancreatic cancer	38
	Lung cancer, large cell (24), adeno (68), small cell (17) and squamous cell (25)	134	Stomach cancer	50
	Oral cavity cancer	56	Renal cell cancer	59
	Breast cancer, ductal (26) and lobular (27)	53	Prostate cancer	48
	Endometrium carcinoma	31	Testis cancer, seminoma (30) and non-seminoma (29)	59
	Ovarian cancer	33	Urinary bladder cancer, non-invasive (pTa 27) and invasive (\geq T2 28)	55
	Uterus cervix carcinoma	28	Thyroid gland cancer	40
	Vulva cancer	39	Leiomyosarcoma	42
	Colon cancer	50	Liposarcoma	36
	Esophageal cancer, squamous cell (23) and adenocarcinoma (25)	48		

samples areas with high tumor cell content ($\geq 60\%$ tumor cells) were marked on the slide. A hollow needle was used to take two tissue cylinders (diameter from 0.5 mm) from each tissue block for nucleic acid isolation. The local ethics committee approved usage of the human tissue samples for research purposes.

RNA extraction and cDNA synthesis. Punched tissue material was deparaffinized with xylene and 80% ethanol. After digestion with proteinases K at 56°C overnight, total RNA was isolated using the RNeasy FFPE kit (Qiagen) in a full-automated nucleic acid isolation device (QIAcube, Qiagen). cDNA was synthesized in a 96-well plate format using the high-capacity cDNA reverse transcription kit (Applied Biosystems) following the manufacturer's instructions. Total RNA (1 μ g) was used for reverse transcription of all samples.

Quantitative PCR analysis. Real-time PCR was performed using the LightCycler LC480 (Roche) detection system, and the QuantiTect SYBR-Green PCR Kit (Qiagen). For specific

amplification of *TPD52* and the housekeeping gene *TBP* the QuantiTect Primer Assay (Qiagen) was used. The following conditions were used for PCR: i) initial denaturation step at 95°C for 10 min; and ii) 40 cycles at 95°C for 20 sec and 55°C for 40 sec. Relative quantity of *TPD52* expression in tumor samples was calculated by the $2^{-\Delta\Delta C_t}$ method standardized to *TPD52* expression in corresponding normal tissue. A fold change of 2 was used to determine the frequency of significant *TPD52* regulated cancers.

Results

Technical issue. A total of 894 cancer samples from 29 different tumor types and 40 normal tissue samples from 20 different normal tissue types were included in the analysis (Table I). A total of 105 (10.5%) tumor samples and 3 (7.5%) normal tissue samples were excluded from analysis because either the reference gene *TBP* or the target gene *TPD52* showed a C_t value exceeding 35, indicating that too little cDNA was generated for reliable *TPD52* expression analysis.

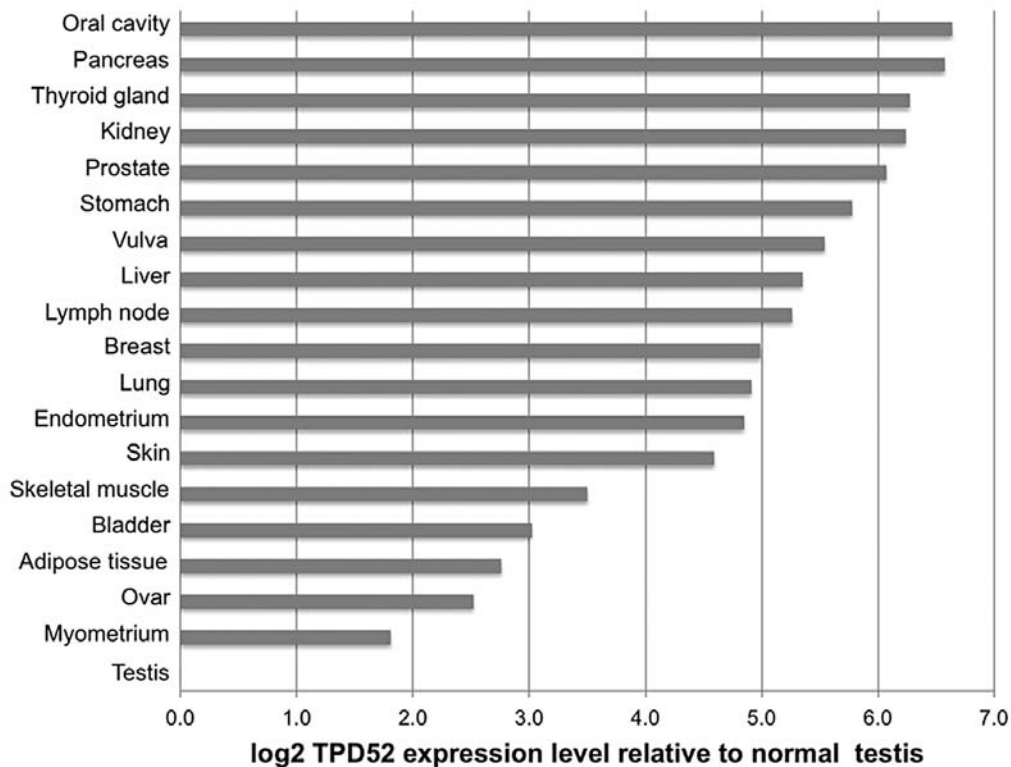


Figure 1. *TPD52* expression levels in different normal tissue. Relative *TPD52* expression level (log2) was standardized to *TPD52* expression in testis.

***TPD52* expression in normal tissues.** *TPD52* transcript was found in all analyzed normal tissues. Average ΔCt was 2.4 ± 0.4 . For a direct comparison of the *TPD52* expression levels of the different normal tissues, the expression levels were normalized to the *TPD52* expression level in testis, which had the lowest expression level of all analyzed normal tissues. Accordingly, highest *TPD52* expression levels [≥ 32 (2^5)-fold overexpression as compared to normal testis] were found in normal oral cavity mucosa (104-fold overexpression), pancreas (91-fold), thyroid gland (79-fold), kidney (76-fold), prostate (69-fold), stomach (56-fold), vulva (45-fold), liver (39-fold), lymph node (38-fold), and breast (32-fold). All data are summarized in Fig. 1.

***TPD52* expression in cancer.** To compare *TPD52* expression across the different tumor types, average expression levels in the cancer samples were normalized to the corresponding normal tissue. For cancer types without available corresponding normal tissues, the average expression level of all normal tissues ($\Delta Ct = 2.4 \pm 0.4$) was used for normalization. These cancer types included tumors of the larynx, cervix, esophagus and colon. This analysis revealed ≥ 1.5 -fold *TPD52* overexpression in 18/29 (62%) analyzed tumor types, with highest levels in non-seminoma (56-fold overexpression compared to corresponding normal tissue), seminoma (42-fold), ductal breast cancer (28-fold) and lobular breast cancer (14-fold). Downregulation as compared to the corresponding normal tissues was found in 11 (38%) of the analyzed tumor types, including papillary renal cell cancer (-8-fold), leiomyosarcoma (-6-fold), clear cell renal cell cancer (-5-fold), liposarcoma (-5-fold) and lung cancer (-4-fold) as the tumor types with the strongest downregulation. All data are summarized in Fig. 2.

Prevalence of TPD52 expression in cancer. In order to estimate the variability of *TPD52* expression in the analyzed tumor types, we determined the fraction of samples showing at least 2-fold up- or downregulation. This analysis revealed that *TPD52* overexpression was particularly frequent (80% of samples showing ≥ 2 -fold overexpression) in germ cell tumors (non-seminoma and seminoma), bladder cancer, esophageal carcinoma and mamma carcinoma, whereas cancer types typically showing *TPD52* downregulation included renal cell carcinoma (90% papillary and 68% clear cell), leiomyosarcoma (69%) and liposarcoma (52%). All data are shown in Fig. 3.

Discussion

The results of our study show that *TPD52* is overexpressed in a multitude of human solid cancer types. Lower *TPD52* levels were also found in the corresponding normal tissues, which is in concordance with previous studies reporting *TPD52* expression in normal tissues derived from breast (25-28), prostate (29-31), ovarian (8), lung (32-34), bladder (35), brain (36), thyroid (37), endometrium (38,39), adrenal gland (40) and liver (41). Such low-level expression was expected given the essential role of *TPD52* for vesicle trafficking and exocytotic secretion (14).

Many cancer types analyzed in our study were characterized by massive *TPD52* overexpression. Overexpression was strongest and also most prevalent in breast cancers, urinary bladder cancers and in testicular cancers. Our data are in agreement with previous studies reporting *TPD52* overexpression in breast cancer [7-47%, (3,42-50)]; bladder cancer [21%, (4)], prostate cancer [44-68%, (2,13,29)] and

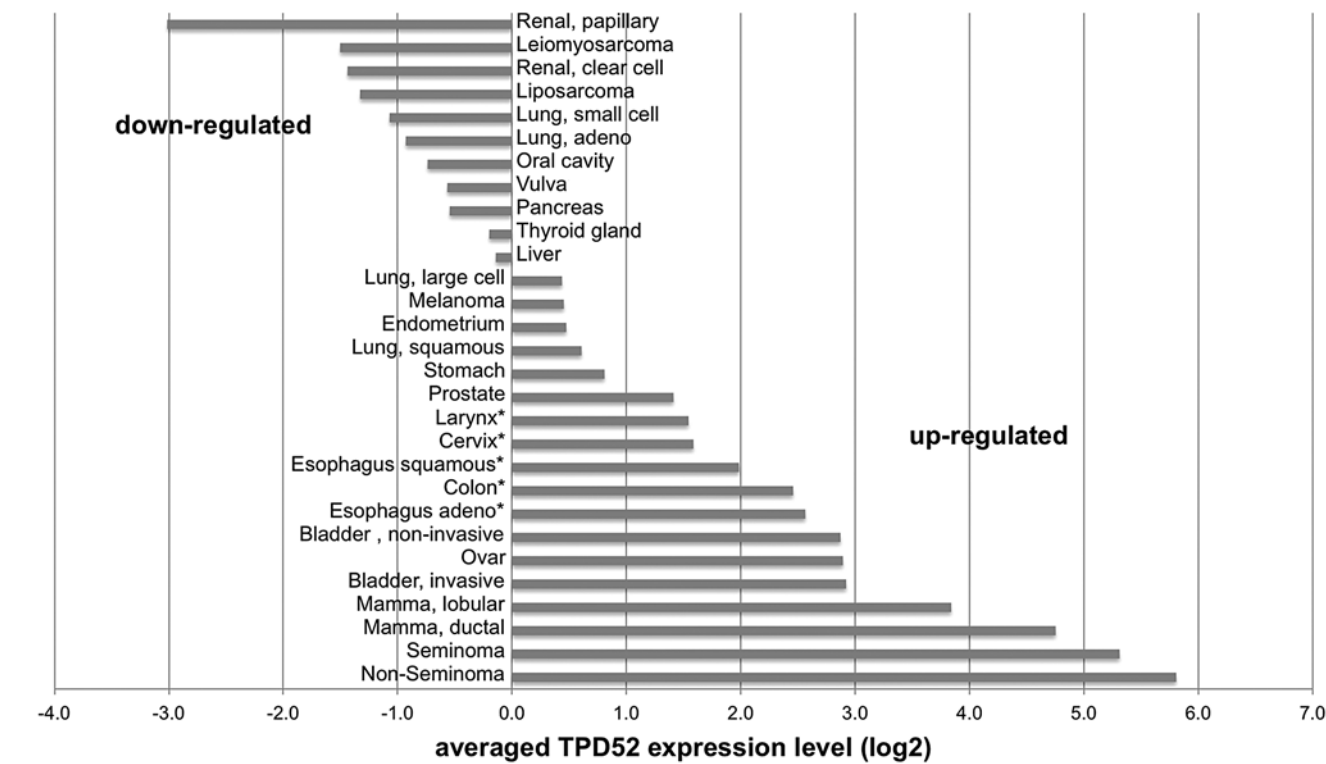


Figure 2. *TPD52* averaged expression levels in different cancer types. *TPD52* expression was normalized to corresponding normal tissue or mean *TPD52* expression in all analyzed normal tissue (*).

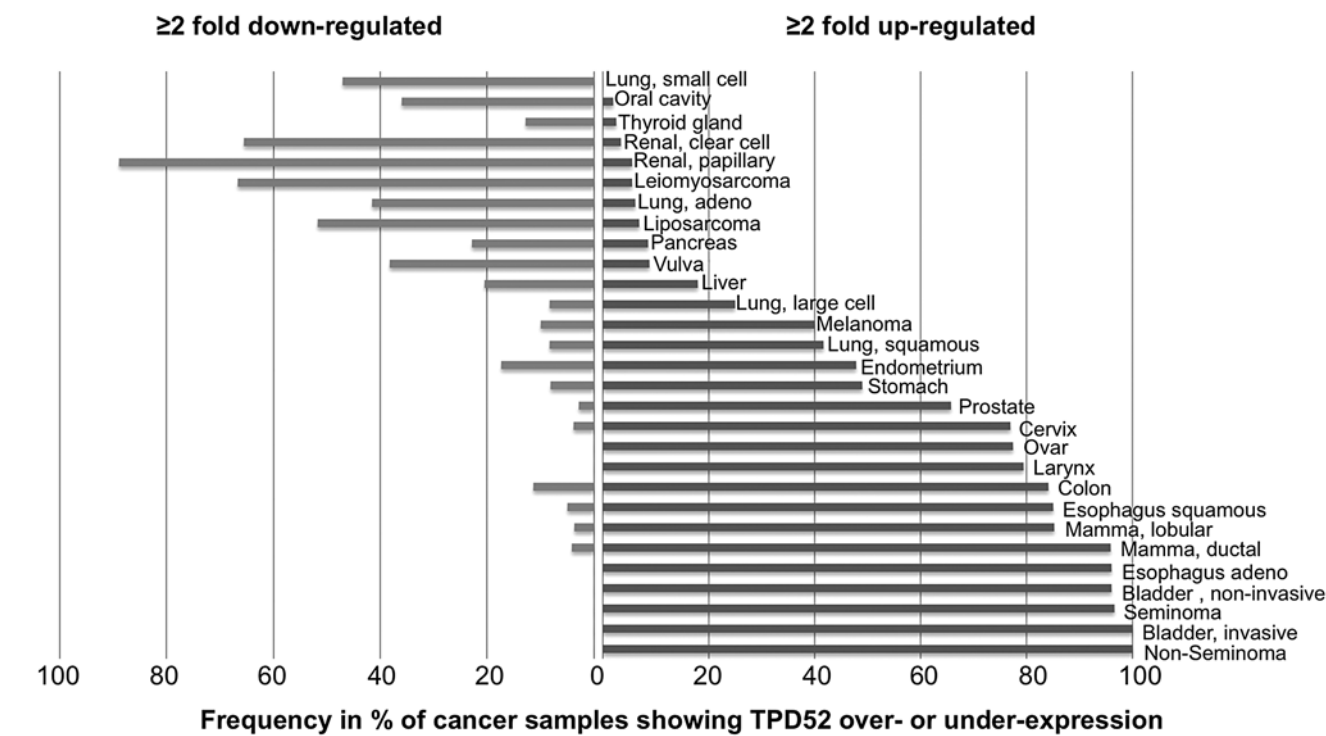


Figure 3. Frequency of *TPD52* up- and downregulation in different tumor types. A fold change of 2 was determined to find significant *TPD52* regulated tumors.

ovarian cancer [61%, (8)] (Table II). Of note, these tumor types are frequently affected by gains of 8q or even high-level amplification of the *TPD52* locus at chromosome 8q21 (3,29),

suggesting that at least part of the observed overexpression in these cancers may be driven by genomic copy number gains of *TPD52*. In line with this assumption, numerous previous

Table II. Overview of cancer types with differential *TPD52* expression from the literature.

Tumor type	TPD52 downregulation (Refs.)	TPD52 overexpression (Refs.)
Malignant melanoma		(20,67 ^a)
Larynx carcinoma		
Lung cancer		(32 ^a ,33 ^a ,68 ^a ,72 ^a)
Oral cavity cancer		
Breast cancer		(9,11,12,22,25, 26,42,43,73-75)
Endometrium carcinoma		
Ovarian cancer		(8,76-78 ^a)
Uterus cervix carcinoma		
Vulvar cancer		
Colon cancer	(61 ^a ,62)	(77-81 ^a)
Esophageal cancer		
Liver cancer		(82 ^a)
Pancreatic cancer		(16)
Stomach cancer		
Renal cell cancer		
Prostate cancer		(2,10,13,24,83-88)
Testis cancer		(21 ^a ,63 ^a ,64 ^a)
Urinary bladder cancer		(89 ^a ,90 ^a)
Thyroid gland cancer		
Leiomyosarcoma		
Liposarcoma		

^aReferences are Oncomine™ analysis for *TPD52* using the following criteria: $p < 0.01$, at least 2-fold differential expression.

studies reported associations between *TPD52* overexpression and gene copy number increase in breast (9,12,42,43,45), prostate (2) and ovarian cancers (8).

We found substantial *TPD52* downregulation in papillary and clear cell kidney cancers, leio- and liposarcomas. Since these cancers do not typically show genomic loss of 8q, it seems obvious that downregulation is not primarily due to *TPD52* deletions but may be a result of modified transcription due to genetic or epigenetic regulation. *TPD52* downregulation was also reported from leukemia (51-53), lymphoma (54,55), brain cancer (36,56-58) and sarcoma (59).

The good concordance of our results as compared to published studies underlines the validity of our analysis. Only few tumor types analyzed in our study showed discrepant results as compared to the literature, including pancreatic cancer, melanoma and colon cancer. It is possible, that these discrepancies are due to technical differences in the first place. For example, Loukopoulos *et al* (16) demonstrated upregulation of *TPD52* in all 42 analyzed tumors, but analyzed xenografts instead of primary tumors in order to artificially maximize the fraction of tumor cells in the sample. Riker *et al* (60), Skrzypczak *et al* (61) and Hong *et al* (62)

reported downregulation of *TPD52* expression in colon cancer, whereas upregulation was found in our analysis. We did not include normal colon as a reference in our study but used an average expression value across all analyzed normal tissues as a surrogate. It is possible, that this strategy resulted in bias. The same may also apply for our results obtained from larynx, cervix and esophageal carcinomas, where we used the same 'average' reference.

The usage of matched normal tissues for reference in the vast majority of our samples enabled us to estimate the relative impact of *TPD52* in the individual tumor types. Testicular germ cell cancers showed the highest levels and frequency of *TPD52* expression, suggesting that *TPD52* upregulation might play a particularly important role in this tumor type. Comparative genomic hybridization data suggest that large fractions of chromosome 8q, including the *TPD52* locus, can be frequently [42-70%, (21,63,64)] gained or amplified (21) in testicular germ cell cancers, supporting a role of genomic gains for the overexpression also in this cancer type. This is consistent with overexpression of *TPD52* in testicular germ cell tumors with CGH-confirmed 8q copy number gains reported by Scothorn *et al* (21).

We identified several additional tumor types with frequent ($\geq 50\%$ of samples) *TPD52* overexpression that had not been reported before, including endometrial carcinomas, cervix cancer, and stomach cancers. Also these cancers are frequently affected by 8q gains or even amplifications (5), further supporting the concept of *TPD52* copy number alterations represent an important mechanism of *TPD52* overexpression (Fig. 3).

The large number of tumor types showing *TPD52* overexpression in a significant fraction of samples encourages clinical testing of anti-*TPD52* therapies. Payton *et al* have shown that *TPD52* protein-based vaccination in mice induced an adaptive immune response capable of rejecting *TPD52*-overexpression induced tumorigenesis (65). Lewis *et al* have shown that TRAMP (transgenic adenocarcinoma of the mouse prostate) mice immunized with cDNA for mD52 as a DNA-based vaccine survived tumor cell challenge through a specific T_H1-type T cell response (66). Our data suggest that such a therapy, if effective, could be applied also to a broad range of other tumor types. This is particularly true for cancers with strong overexpression in the tumor cells as compared to the corresponding normal tissue, including for example testicular germ cell cancers. Our finding, that breast cancer ranks second as a tumor type with strong cancer-related *TPD52* expression level changes emphasized the potential of anti-*TPD52* therapy in this tumor type.

In summary, our data demonstrate that *TPD52* overexpression is common in many tumor types. Particularly strong *TPD52* upregulation was found in cancers of the breast, urinary bladder cancer and testicular germ cell cancers, which frequently harbor 8q gains. These tumor types may be particularly promising candidates for potential anti-*TPD52* therapies.

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