Histol Histopathol (2022) 37: 739-748

http://www.hh.um.es

Histology and Histopathology
From Cell Biology to Tissue Engineering

ORIGINAL ARTICLE **Open Access**

DOG1 overexpression is associated with mismatch repair deficiency and BRAF mutations but unrelated to cancer progression in colorectal cancer

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Summary. Introduction. The transmembrane channel protein DOG1 (Discovered on GIST1) is normally expressed in the gastrointestinal interstitial cells of Cajal and also in gastrointestinal stroma tumors arising from these cells. However, there is also evidence for a relevant role of DOG1 expression in colorectal cancers. This study was undertaken to search for associations between DOG1 expression and colon cancer phenotype and key molecular alterations.

Methods. A tissue microarray containing samples from more than 1,800 colorectal cancer patients was analyzed by immunohistochemistry.

Results. DOG1 immunostaining was detected in 503 (30.2%) of 1,666 analyzable colorectal cancers and considered weak in 360 (21.6%), moderate in 78 (4.7%), and strong in 65 (3.9%). Strong DOG1 immunostaining was associated with advanced pT stage (p=0.0367) and nodal metastases (p=0.0145) but these associations were not retained in subgroups of 1,135 mismatch repair proficient and 86 mismatch repair deficient tumors. DOG1 positivity was significantly linked to several molecular tumor features including mismatch repair deficiency (p=0.0034), BRAF mutations (p<0.0001), nuclear p53 accumulation (p=0.0157), and PD-L1 expression (p=0.0199) but unrelated to KRAS mutations and the density of tumor infiltrating CD8 positive lymphocytes.

Conclusion. Elevated DOG1 expression is frequent in colorectal cancer and significantly linked to important molecular alterations. However, DOG1 overexpression is largely unrelated to histopathological parameters of cancer aggressiveness and may thus not serve as a prognostic parameter for this tumor entity.

Key words: DOG1, Colon Cancer, Tissue micro array, Immunohistochemistry

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DOI: 10.14670/HH-18-475

Introduction

Colorectal cancer was the third most common cancer worldwide in 2018 and the second most common cause for cancer related death (Bray et al., 2018). Standard treatment of colorectal cancer consists of surgical removal. In high-risk cancers adjuvant chemotherapy is also given in order to destroy micro-metastasis and to reduce the possibility of local recurrence. Possible chemotherapies include conventional cytotoxic chemotherapy and several antiangiogenic substances. In the case of BRAF, KRAS and NRAS wild type cancers anti-EGFR therapies antibodies can also be applied (summarized in (Afrasanie

et al., 2019)). Immune checkpoint inhibitors can be administered in cancers harboring microsatellite instability (MSI) or mismatch repair deficiency (dMMR) (summarized in (Sahin et al., 2019)). Established prognostic factors of colorectal carcinomas include pathological tumor stage (pT), pathological lymph node status (pN), status of distant metastasis (M) and histologic tumor features (Fleming et al., 2012; Amin et al., 2017). These are statistically powerful but cannot reliably predict disease course in individual patients.

DOG1 (Discovered On Gastrointestinal Stromal Tumors Protein 1, GIST1), also known as Transmembrane Protein 16A (TMEM16A) or Anoctamin-1 (ANO1) is a voltage-gated calcium-activated chloride and bicarbonate channel (Caputo et al., 2008; Yang et al., 2008). DOG1 is highly expressed in the gastrointestinal interstitial cells of Cajal, where it plays an important role in epithelial chloride secretion mediating intestinal motility (Miettinen et al., 2009; Chevalier et al., 2020). Calcium-activated chloride channel blocking drugs like niflumic acid have been shown to block slow waves (pacemaker activity) - which produce motility - in the human small intestine and stomach (Hwang et al., 2009). High levels of DOG1 expression are a diagnostic hallmark of gastrointestinal stromal tumors, a tumor derived from interstitial cells of Cajal (Kindblom et al., 1998; Sircar et al., 1999; West et al., 2004; Miettinen et al., 2009). However, DOG1 expression was also reported to occur in colorectal cancer. Foda and Mohamed (2015) reported DOG1 immunostaining in 10% of 150 cancers and found no association with patient outcome or unfavorable tumor phenotype. In contrast, Jiang et al. (2019) recently found high DOG1 expression by immunohistochemistry (IHC) in 63.9% of 122 tumors and reported a strikingly worse patient outcome in high expressors. In line with this observation, these authors also observed a suppression of aggressive tumor behavior of cell lines by targeting DOG1. Evidence for a functionally active role of DOG1 in cancer cells was also provided from studies on pancreatic and colorectal cancer cell lines demonstrating that a diminished activity of DOG1 attenuated migration, invasion, and proliferation and promoted cell cycle arrest in G0/G1 phase *in vitro* (Sui et al., 2014, 2015). Overall, these data make DOG1 an interesting prognostic parameter and a potential therapeutic target in colorectal cancer.

To learn more on the role of DOG1 in colorectal adenocarcinoma, we searched for associations between DOG1 expression and colon cancer phenotype and its key molecular alterations, including BRAF and RAS mutations, microsatellite instability, PD-L1 expression and cytotoxic T-cell (CD8+) density in a large cohort of more than 1,800 colorectal cancers.

Materials and methods

Tissue microarray (TMA)

Our colon cancer TMA consisted of 1,802 colon cancers diagnosed at the Institutes of Pathology of the University Medical Center Hamburg-Eppendorf (Hamburg, Germany) and the Department of Pathology of the Academic Hospital Fuerth (Fuerth, Germany) between 2009 and 2019. Tumors were not selected for particular pathological or clinical features but taken consecutively from the archives of the Pathology Departments. TMA construction was done as previously described (Kononen et al., 1998; Dancau et al., 2016). The available clinical, pathological and molecular parameters were obtained from patient records (Table 1). No data were available on patient prognosis or therapy. Data on KRAS (exons 12, 13 and 61) and BRAF (exon 15) mutation were obtained by Sanger sequencing during routine pathological examination and were taken from the patient files. The use of archived remnants of diagnostic tissues for manufacturing of tissue microarrays and their analysis for research purpose as well as patient data analysis were approved by local laws (HmbKHG, §12) and by the local ethics committee (Ethics Commission Hamburg, WF-049/09). All work was carried out in compliance with the Helsinki Declaration.

Immunohistochemistry (IHC)

Freshly prepared TMA sections were immunostained on one day in one experiment. Slides were deparaffinized with xylol, rehydrated through a graded alcohol series and exposed to heat-induced antigen retrieval for 5 minutes in an autoclave at 121°C in pH

Table 1. Patient cohort.

	all tumors (n=1,802)
age	
median	73.2
mean	72.1
tumor localisation	
caecum	172 (9.6%)
c. ascendens	200 (11.2%)
c. transversum	110 (6.2%)
c. descendens	115 (6.5%)
c. sigmoideum	725 (40.7%)
rectum	461 (25.9%)
colon side	
left	1311 (73.1%)
right	483 (26.9%)
tumor stage	
pT1	76 (4.3%)
pT2	354 (19.8%)
pT3	989 (55.4%)
pT4	365 (20.5%)
lymph node status	
pN-	926 (52.4%)
pN+	841 (47.6%)
mismatch repair status	
deficient	94 (7.2%)
proficient	1203 (92.3%)

c: colon.

7.8 DakoTarget Retrieval Solution™ (Agilent, CA, USA; #S2367). Endogenous peroxidase activity was blocked with Dako Peroxidase Blocking Solution™ (Agilent, CA, USA; #52023) for 10 minutes. Primary antibody specific against DOG1 protein (mouse monoclonal, dilution 1:150, MSVA-201M, MS Validated Antibodies, Hamburg, Germany) and p53 (DO7, mouse monoclonal, dilution 1:150, M7001, Agilent, CA, USA) was applied at 37°C for 60 minutes. The bound antibody was then visualized using the EnVision Kit™ (Agilent, CA, USA; #K5007) according to the manufacturer's directions. The sections were counterstained with haemalaun. DOG1 staining was membranous but sometimes accompanied by weaker cytoplasmic staining. p53 staining was nuclear. The staining for both was interpreted as follows: Negative: no staining in tumor cells, weak: staining intensity of 1+ in ≤ 70% of tumor cells or 2+ in ≤ 30% of tumor cells, moderate: staining intensity of 1+ in > 70% of tumor cells, 2+ in > 30% but in ≤ 70% of tumor cells or 3+ in ≤ 30% of tumor cells, strong: staining intensity of 2+ in > 70% of tumor cells or 3+ in > 30% of tumor cells. Data on the expression of the MMR proteins MLH1, PMS2, MSH2, and MSH6, p53, and PD-L1 as well as the density of CD8 positive cytotoxic lymphocytes were available from earlier studies using the same set of TMAs (Blessin et al., 2021; Moller et al., 2021; Rico et al., 2021).

Statistics

Statistical calculations were performed with JMP® software (SAS Institute Inc., NC, USA). Contingency tables and the chi^2-test were performed to search for associations between DOG1 expression, clinical-pathological and molecular parameters. ANOVA test was used to examine for differences in the density of CD8 positive cells between tumor categories. A p-value ≤0.05 was regarded as statistically significant.

Results

Technical issues

The DOG1 expression analysis was informative in 1,666 (92.5%) of the 1,802 arrayed cancers. Reasons for non-informative cases included lack of tissue samples or absence of unequivocal cancer cells in the TMA spot.

DOG1 expression, tumor phenotype and molecular

Table 2. DOG1 immunostaining and histological and molecular features of colon cancer.

			DOG1 immunostaining result				
		n	negative (%)	weak (%)	moderate (%)	strong (%)	p
all cancers		1666	69.8	21.6	4.7	3.9	
Tumor stage	pT1	68	69.1	19.1	10.3	1.5	0.0367
	pT2	332	67.8	22	6.9	3.3	
	pT3	916	72.1	21	2.9	4	
	pT4	335	67.2	22.7	6	4.2	
Lymph node status	pN-	859	73.3	18.5	4.7	3.5	0.0145
	pN+	777	66.4	24.7	4.8	4.1	
Blood vessel invasion	V0	1200	69.3	21.3	5	4.3	0.1908
	V+	425	70.6	23.1	4	2.4	
Lymph vessel invasion	L0	610	71.1	20.8	4.1	3.9	0.7102
	L1	1003	68.9	22.1	5.1	3.9	
Tumor localization	left colon	1204	70.7	21.7	4.3	3.3	0.1641
	right colon	455	67.7	21.3	5.5	5.5	
Mismatch repair status	defecient	86	67.4	14	10.5	8.1	0.0034
	proficient	1135	69.3	23.6	4	3.2	
RAS mutation	present	345	68.4	23.8	2.9	4.9	0.2498
	absent	446	68.8	22.9	5.2	3.1	
BRAF V600E mutation	present	21	19	47.6	9.5	23.8	<0.0001
	absent	126	73.8	17.5	6.3	2.4	
p53 IHC result	negative	268	66	26.9	4.9	2.2	0.0157
	weak	291	67	21	5.8	6.2	
	moderate	123	69.1	26	0.8	4.1	
	strong	674	72.7	20.8	3.7	2.8	
PD-L1 IHC result (tumor cells)	negative	1227	72.1	21.6	3.6	2.7	0.0199
	positive	52	57.7	26.9	3.8	11.5	
CD8+ T cell density (cells/mm^2)		1584	251±14	288±25	304±54	213±59	0.3942

IHC: immunohistochemistry.

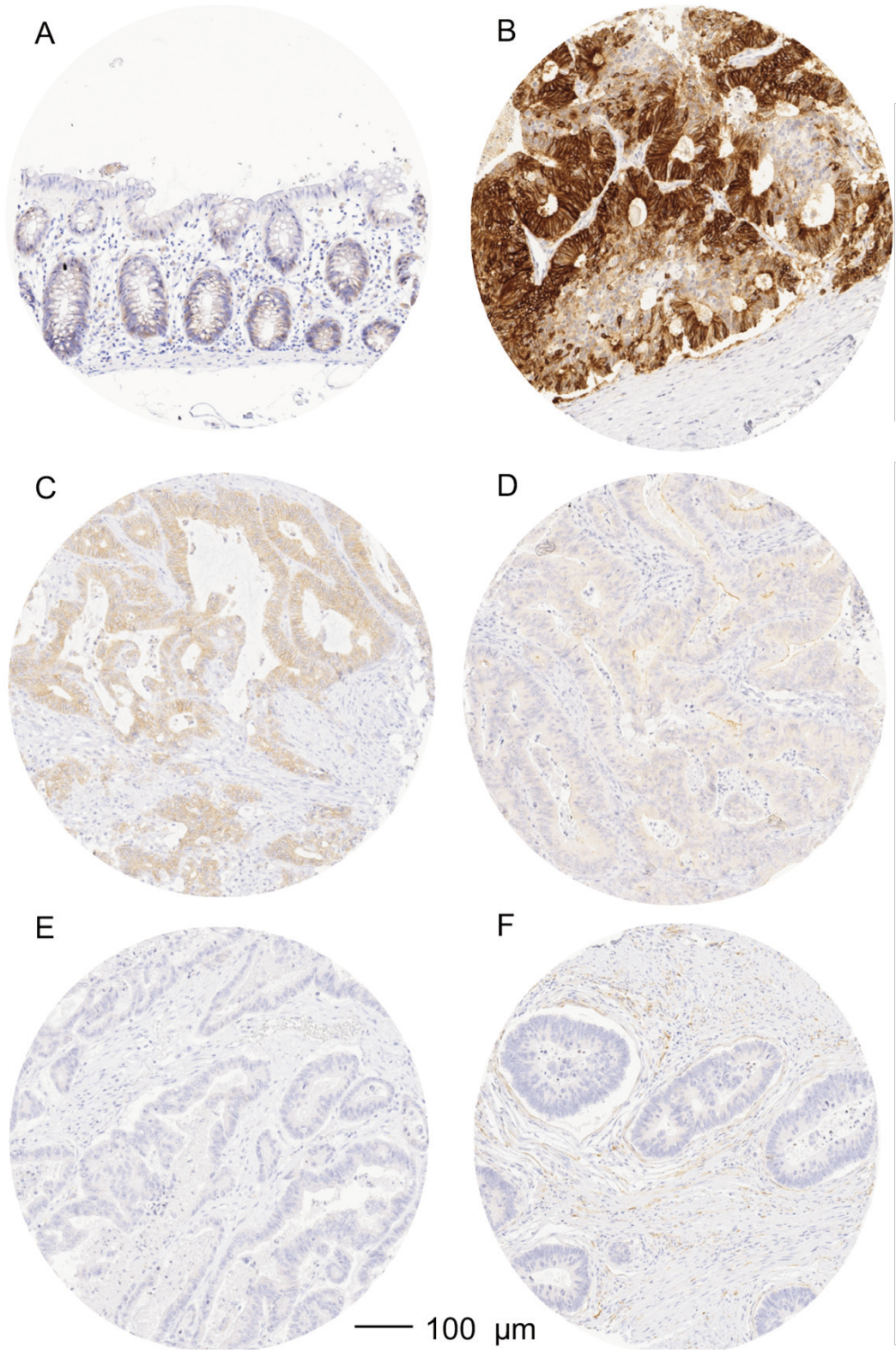

Fig. 1. DOG1 immunostaining. The panels show a weak membranous DOG1 staining of few cells located at the base of crypts of the normal colon **(A)**, and a strong **(B)**, moderate **(C)**, and weak **(D)** DOG1 immunostaining in cancers. The staining is limited to the apical membrane in **D**. The panels **E** and **F** show DOG1 negative cancers with a distinct stroma cell staining occurring in **F**.

tumor features

In the normal colon, a weak to moderate DOG1 staining was seen at the apical membranes of epithelial cells at the base of crypts (Fig. 1A). In colorectal cancer, a membranous DOG1 staining was seen in 503 (30.2%) of 1,666 analyzable tumor spots. The staining patterns varied from variable numbers of interspersed DOG1 positive cells, patchy focal staining, and intense diffuse positivity. According to our classification, positive cases included 360 (21.6%) cancers with weak (Fig. 1D), 78 (4.7%) with moderate (Fig. 1C), and 65 (3.9%) with strong DOG1 positivity (Fig. 1B). An example of negative staining is given in Fig. 1E. In a fraction of cases, a DOG1 immunostaining could also be observed in the stroma cell (Fig. 1F). Strong DOG1 positivity was significantly associated with advanced pT category (p=0.0367), nodal metastasis (p=0.0145), dMMR (p=0.0034), BRAF mutations (p<0.0001), p53 positivity (p=0.0157), and PD-L1 expression in tumor cells (p=0.0199; Table 2). No association was seen between the number of tumors infiltrating CD8 positive T lymphocytes and the level of DOG1 expression (p=0.3942). Because of the fundamental prognostic and molecular differences between colorectal carcinomas with and without mismatch repair deficiency, these tumor subsets were separately analyzed for associations between DOG1 and histopathological and molecular tumor features (Table 3). The analysis of 1,135 mismatch repair proficient (pMMR) tumors now failed to find significant associations between DOG1 staining and pT (p=0.0986) and pN categories (p=0.1472), but associations with molecular features such as p53 status (p=0.0402), BRAF mutations (p<0.0001), and PD-L1 expression in tumor cells (p=0.0024) were retained. Significant associations between DOG1 expression and any of the examined histopathological and molecular tumor features were not found in the subgroup of 86 dMMR tumors.

p53 immunostaining

IHC analysis of p53 was informative in 1,443 (80.1%) of the 1,802 arrayed colorectal cancers. A nuclear p53 immunostaining indicates presence of inactivating p53 mutations that alter protein half-life and result in nuclear accumulation of the defective protein (Esrig et al., 1993; Nakayama and Oshima, 2019; Quinn et al., 2019). Nuclear p53 immunostaining was seen in 1,159 (80.3%) of the 1,443 analyzable tumor spots, including 313 (21.7%) with weak, 130 (9.0%) with moderate, and 716 (49.6%) with strong staining.

Table 3. DOG1 in mismatch repair proficient and deficient colon cancers.

		Mismatch repair proficient tumors (pMMR)						Mismatch repair deficient tumors (dMMR)					
			DOG1 immunostaining result						DOG1 immunostaining result				
		n	negative (%)	weak (%)	moderate (%)	strong (%)	p	n	negative (%)	weak (%)	moderate (%)	strong (%)	p
all cancers		1135	69.3	23.6	4	3.2		86	67.4	14	10.5	8.1	
Tumor stage	pT1	44	68.2	22.7	9.1	0	0.0986	5	60	0	40	0	0.4936
	pT2	234	66.7	26.1	4.7	2.6		19	63.2	15.8	15.8	5.3	
	pT3	624	71.6	22.4	2.4	3.5		43	67.4	16.3	4.7	11.6	
	pT4	222	67.1	23.9	6.3	2.7		19	73.7	10.5	10.5	5.3	
Lymph node stage	pN-	578	72.5	20.9	3.8	2.8	0.1472	56	71.4	14.3	7.1	7.1	0.4867
	pN+	536	66.2	26.3	4.1	3.4		29	58.6	13.8	17.2	10.3	
Blood vessel invasion	V0	805	68.3	23.7	4.3	3.6	0.2278	71	64.8	15.5	11.3	8.5	0.7452
	V+	300	71	24.3	3	1.7		14	78.6	7.1	7.1	7.1	
Lymph vessel invasion	L0	452	70.8	23.2	3.3	2.7	0.5932	39	59	17.9	10.3	12.8	0.2641
	L1	640	68	24.1	4.5	3.4		45	75.6	8.9	11.1	4.4	
Tumor localization	left colon	872	69.6	23.7	3.7	3	0.7851	37	73	13.5	8.1	5.4	0.7342
	right colon	257	68.5	23	4.7	3.9		49	63.3	14.3	12.2	10.2	
RAS mutation	present	279	68.5	25.8	1.8	3.9	0.0539	8	62.5	0	25	12.5	0.4026
	absent	356	71.1	23.3	4.2	1.4		21	47.6	14.3	14.3	23.8	
BRAF V600E mutation	present	9	0	77.8	22.2	0	<0.0001	6	16.7	16.7	0	66.7	0.0538
	absent	107	72.9	19.6	5.6	1.9		10	60	10	20	10	
p53 IHC result	negative	207	67.1	28	3.9	1	0.0402	10	80	10	10	0	0.3212
	weak	193	66.3	22.3	5.2	6.2		41	70.7	12.2	12.2	4.9	
	moderate	89	66.3	28.1	1.1	4.5		13	84.6	7.7	0	7.7	
	strong	557	72	21.7	3.8	2.5		13	46.2	23.1	7.7	23.1	
PD-L1 IHC result (Tumor)	negative	907	72.1	22.4	3.2	2.3	0.0024	53	71.7	11.3	11.3	5.7	0.6781
	positive	24	41.7	45.8	0	12.5		13	61.5	15.4	7.7	15.4	
CD8+ T cell density (cells/mm^2)		1095	246±16	312±28	349±68	167±78	0.0762	83	439±76	461±165	426±202	630±216	0.8696

Discussion

A positive DOG1 immunostaining was found in 30.2% of colorectal adenocarcinomas in this study. This positivity rate is in the middle range of prevalence data from four previous studies reporting DOG1 positivity in 1 (5%) of 20 (Miettinen et al., 2009), 15 of 150 (10%) (Foda and Mohamed, 2015), 3 of 10 (30%) (Hemminger and Iwenofu, 2012), and in 78 of 122 (64% high expression) (Jiang et al., 2019) of colorectal adenocarcinomas. Typical reasons for discrepant results include the use of different antibodies, IHC protocols, and cut-off levels or scores to categorize a staining as "positive" as well as the composition of the tumor cohorts. Different antibodies can lead to different results in the case of cross-reactivity with other proteins. For example, we have earlier compared staining patterns of MSVA-201M with that of another frequently used anti-DOG1 antibody (clone SP31) and found that SP31, but not MSVA-201M, shows non-specific staining of a protein that is present in spermatocytes (Jansen et al., 2021). Different IHC protocols, for example a different antibody dilution, can obviously be expected to change the overall rate of positive cancers. With respect to the composition of the tumor cohort, Foda and Mohamed (2015) had indeed enriched their tumor cohort for mucinous carcinomas which made up for 50% of their 150 tumors. As no significant differences were seen in the rate of DOG1 expression between mucinous carcinomas and randomly selected group of non-mucinous carcinomas, patient selection might not have impacted the overall DOG1 expression prevalence in this cohort of tumors. The authors employed the prediluted ready to use monoclonal rabbit antibody clone SP31 (Cell Marque) for their study (Foda and Mohamed, 2015). Hemminger et al. found a focal, predominantly luminal staining of three of 10 cancers by using the antibody clone K9 (Hemminger and Iwenofu, 2012), which was also used by Miettinen et al. (2009). In the study by Jiang et al. (2019) an unselected consecutive cohort of patients was analyzed by using a polyclonal rabbit anti-human DOG1 antibody sc-377115 (Santa Cruz Biotechnology).

Although more than 1,600 cancers were successfully analyzed in our study, clear-cut associations with histopathological parameters of high cancer aggressiveness were not found. This is in agreement with the results by Foda and Mohamed (2015) who also failed to find a link between DOG1 and advanced tumor stage or nodal metastasis, in contrast to the data from Jiang et al. (2019) reporting striking prognostic differences between 44 cancers with low and 78 cancers with high DOG1 expression. Data from several experimental models provided evidence for DOG1 upregulation resulting in increased cancer cell aggressiveness (Duvvuri et al., 2012; Britschgi et al., 2013; Godse et al., 2017; Wang et al., 2019) and DOG1 suppression resulting in decreased cancer cell viability (Duvvuri et al., 2012; Britschgi et al., 2013; Godse et al., 2017; Crottes et al., 2019; Hu et al., 2019; Wang et al., 2019; Yu et al.; 2019). *In vivo*, DOG1 overexpression was associated with larger tumor size and DOG1 depletion/inhibition with decreased tumor growth in several cancer models (Duvvuri et al., 2012; Godse et al., 2017; Song et al., 2018; Hu et al., 2019; Wang et al., 2019; Yu et al., 2019). A total of 16 studies had so far analyzed the prognostic role of DOG1 expression in cancer. 11 of them described evidence for poor prognosis or aggressive tumor phenotype in cancers with high DOG1 expression in GIST, hepatocellular carcinomas, carcinomas of the prostate, breast, stomach, ovarian, and pancreas, as well as squamous cell carcinomas of the oral cavity, esophagus, and head and neck (Duvvuri et al., 2012; Liu et al., 2012; Li et al., 2014; Liu et al., 2015, 2019; Godse et al., 2017; Sahin et al., 2017; Bae et al., 2018; Crottes et al., 2019; Yu et al., 2019; Zeng et al., 2019). Four studies failed to find such associations in GIST and colorectal cancers (Peng et al., 2013; Foda and Mohamed, 2015; Kisluk et al., 2016; Varshney et al., 2019). Moreover, one study has described an association of high DOG1 staining and lower stage and favorable prognosis in breast cancer (Wu et al., 2015).

The molecular data that were previously collected for the tumors of our TMA enabled us to interrogate the relationship of DOG1 expression with various other parameters of interest. For this study, we selected mismatch repair deficiency because it represents the therapeutically and prognostically most relevant molecular alteration in colorectal cancer (summarized in Sahin et al. (2019), some of the most commonly mutated genes (KRAS, BRAF, p53) (summarized in Bahrami et al., 2018; Bonnot and Passot, 2019; Malki et al., 2020), as well as the expression of PD-L1 and the density of CD8 positive tumor infiltrating lymphocytes as potential markers for the "immune status" of the tumor (Craig et al., 2020). The impact of these important molecular alterations on DOG1 expression so far have not been investigated in colorectal cancer nor in other tumors. That strong statistical associations were found between DOG1 expression levels and most of these molecular parameters suggests that DOG1 expression is dependent on several key molecular pathways in colorectal carcinoma. As experimental artifacts in immuno-histochemistry are unlikely to result in multiple significant statistical associations – especially in comparison to results obtained by other methods such as DNA sequencing – these findings also constitute a validation of our experimental approach.

The most striking link was observed between BRAF mutations and DOG1 overexpression. 23.8% of BRAF mutated cancers showed strong DOG1 expression as compared to 2.4% of non-mutated tumors. This finding is in line with one functional study showing upregulation of DOG1 expression via activation of the EGFR/STAT3 signaling pathway (Wang et al., 2019), in which BRAF acts as a molecular on/off switch. Furthermore, it is known that the activating BRAF mutation V600E leads to an EGFR independent and permanent activation of the

EGFR pathway (summarized in Nakayama et al., 2020). That high DOG1 expression was linked to dMMR - typically accompanied by a high number of DNA mutations - but inversely correlated with nuclear p53 accumulation (which indicates inactivating p53 mutations) (Esrig et al., 1993; Nakayama and Oshima, 2019; Quinn et al., 2019) which is a feature of tumors with elevated risk for double strand DNA breakage may suggest that these two mechanisms for genomic instability exert inverse effects on DOG1 expression or that these mechanisms have no direct impact on DOG1 expression. There are three major pathways that can lead to the development and progression of colorectal carcinomas. The classical adenoma-to-carcinoma pathway characterized by chromosomal instability and TP53 mutations, the loss of mismatch repair mechanism by germline mutations of mismatch repair genes, and the serrated/methylator pathway characterized by BRAF mutations. The latter is also associated with MSI (Clarke and Kopetz, 2015). The strong association of DOG1 with BRAF mutations and MSI and inverse correlation with TP53 mutations suggests that DOG1 overexpression is a marker for tumors that have developed via the serrated/methylator pathway. That DOG1 expression levels were unrelated to the density of CD8 positive T-lymphocytes argues against a particular impact of DOG1 overexpression on tumor immunogenicity or a tumors capability of evading anti-tumor immune response. The particularly high PD-L1 expression in DOG1 positive cancers may suggest, however, that PD-L1 expression is a preferred mechanism for immune evasion in case of high level DOG1 expression. It is of note that the link between DOG1 overexpression and the prognostically unfavorable PD-L1 up-regulation did not translate into an overall unequivocal association between DOG1 overexpression and colon cancer progression in our study. This suggests complex interactions between the various molecular alterations involved in colon cancer progression that still need to be elucidated. It will be interesting to learn more about the functional role of DOG1 in cell line models harboring defined key mutations of colorectal cancers.

It is of note that - based on the general role of DOG1 overexpression in tumorigenesis and progression - DOG1 may also represent a suitable drug target. *In vitro* and *in vivo* studies have shown that DOG1 inhibition with T16Ainh-A01 and CaCCinh-A01 results in decreased channel activity, tumor cell viability, cell proliferation, cell migration, increased apoptosis, cell cycle arrest in G0/G1 phase, and reduced tumor growth in functional models of GIST and breast, bladder, esophagus, lung, and head and neck carcinomas (Duvvuri et al., 2012; Britschgi et al., 2013; Berglund et al., 2014; Guan et al., 2016; Kulkarni et al., 2017; Frobom et al., 2019; Hu et al., 2019). Furthermore, it was shown that combined inhibition of DOG1 and HER2 or DOG1 and EGFR leads to decreased cell growth in a cooperative manner and that inhibition of DOG1 can counteract EGFR and HER2 therapy resistance *in vitro* and *in vivo* (Bill et al., 2015; Fujimoto et al., 2017; Kulkarni et al., 2017). Given the considerable high numbers of DOG1 positive colorectal cancers, one might speculate that anti DOG1 treatment might be potentially promising in these carcinomas.

In summary, the results of our study show that elevated DOG1 expression is frequent in colorectal cancer and significantly linked to BRAF mutations and other relevant molecular alterations. However, DOG1 overexpression is largely unrelated to histopathological parameters of cancer aggressiveness and may thus not be useful as a prognostic parameter for this tumor entity.

Acknowledgements. We are grateful to Melanie Witt, Inge Brandt, Maren Eisenberg, and Sünje Seekamp for excellent technical assistance.

Statement of Ethics. Utilization of archived remnants of diagnostic tissues for manufacturing of TMAs and their analysis for research purposes as well as patient data analysis has been approved by local laws (HmbKHG, §12) and by the local ethics committee (Ethics commission Hamburg, WF-049/09). All work has been carried out in compliance with the Helsinki Declaration.

Conflict of Interest Statement. The DOG1 antibody clone MSVA-201M was received from MS Validated Antibodies GmbH (owned by a family member of GS).

Funding Sources. No funding was received

Authors Contributions. KJ, MK, CHM, SS, RS, JRI, GS, TK contributed to conception, design, data collection, data analysis and manuscript writing.

FJ, CB, EB, TSC, WW, RU participated in pathology data analysis and data interpretation.

KJ, RS, NCB, AHM, PCR and immunohistochemistry analysis.

MN, HM, HL, TD, CI, SC, DD, HR, AG, DP conception and design, collection of samples.

KJ, RS, GS, TK study supervision.

All authors agree to be accountable for the content of the work, contributed to the research and to the final document.

Data availability statement. Raw data are available upon reasonable request from the corresponding author. All data relevant to the study are included in the article.

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Accepted June 1, 2022