

Expression of DOG1 (Using SP31) in Poorly Differentiated Carcinoma of the Head and Neck

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Abstract. *Background:* The calcium-activated chloride channel protein discovered on gastrointestinal stromal tumour 1 (DOG1) is expressed in a variety of normal and neoplastic tissues. DOG1 is a specific marker for gastrointestinal stromal tumour. In the head and neck region, DOG1 is a sensitive discriminator for acinar cell carcinoma. Only a few publications have presented data concerning the expression of DOG1 in head and neck squamous cell carcinoma (HNSCC). The expression of DOG1 in HNSCC appears to be associated with a poor prognosis. The aim of this study was to analyze the expression pattern of DOG1 in poorly differentiated carcinoma of the upper aerodigestive tract. *Materials and Methods:* A total of 84 specimens from 31 patients with carcinomas of the upper aerodigestive tract were immunohistochemically investigated for DOG1 expression. Inclusion criterion was poorly to undifferentiated carcinoma of the head and neck, but samples of the same resection site that exhibited moderate or well-differentiated squamous cell carcinoma were also enrolled. Immunoreactivity in carcinomas was estimated using a visual score (0: negative; 1: basally positive, 2: parabasally positive, 3: completely positive, 4: basally and parabasally positive). *Results:* Fifteen out of 84 specimens were immunoreactive to antibody to DOG1 (17.8%). DOG1 immunoreactivity was restricted to eight patients (25.8%). However, DOG1 expression was considerably heterogeneous in tumours, with three (9.6%)

cases showing a positive reaction in all samples. Basal and parabasal staining patterns (five specimens each) dominated. *Discussion:* This study demonstrated expression of DOG1 to be restricted to some poorly differentiated carcinomas of the upper aerodigestive tract. Although the proportion of DOG1-positive carcinomas was moderate compared to results of previous studies on head and neck cancer tissues, DOG1 expression possibly indicates a subset of HNSCC. Further studies are necessary to investigate the heterogeneity and clinical relevance of DOG1 expression in HNSCC.

Discovered on gastrointestinal stromal tumours (GIST)-1 (DOG1) is a protein strongly expressed on the cell surface of GISTs (1-5). This antigen is rarely expressed in other soft-tissue tumours (1). However, as well as the high specificity of DOG1 in GISTs, this antigen is also identifiable in some other tumours (6). In the head and neck region, this protein is expressed in some epithelial tumours, in particular in specialized glandular cells (7-9) and also in squamous epithelial cells (10-14). In fact, in addition to GIST diagnostics, identification of this antigen can be used for typing certain salivary gland carcinomas (15). Furthermore, experimental studies provided evidence for DOG1 expression by epithelia during certain phases of mammalian embryogenesis (16, 17). However, the function of this protein is largely unknown (18).

The gene coding for DOG1 is synthesized on chromosome 11q13 (19, 20). Amplification of the chromosomal region around band 11q13 was detected in numerous tumours, including oral squamous cell carcinoma (OSCC) and squamous cell carcinoma of the head and neck (HNSCC) (20-24). The amplification of this region has proven to be relevant in estimating prognosis in some cancer entities (26, 27) and initiated studies to identify in detail the protein-expression profiles of this region in cancer (24).

DOG1 is only one designation among many others to describe this protein (25). Different research groups investigated this protein and discovered that DOG1 belongs to the group of

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Table I. Discovered on gastrointestinal tumour protein 1 (DOG1) immunoreactivity in head and neck carcinoma.

Patient no.	Age (years)	Gender	No. of positive specimens/ no. of specimens	Histological diagnosis	Sampling localization of DOG1-stained tissue	Score	Remarks
1	81	M	1/1	Undifferentiated carcinoma	Nasopharynx	3	
2	67	W	1/3	Undifferentiated solid carcinoma, suspected metastatic breast cancer	Lymph node, neck	1	
3	62	M	1/1	Lymphoepithelial carcinoma	Nasopharynx	2	
4	64	M	3/6	Solid carcinoma (G2)	Lymph node, neck	1, 2 and 4	Salivary gland DOG1 immunoreactive; carcinoma immunoreactivity ranged between 5 and 60%
5	55	M	2/6	Lymphangiosis carcinomatosa	Neck	2 and 4	Carcinoma immunoreactivity was 5% and 70%
6	48 (52)	M	2/2	Lymphoepithelial carcinoma	Naso- and hypopharynx (metachronous)	3 (both)	Carcinoma immunoreactivity was 30% and 80%
7	45	M	3/9	Squamous cell carcinoma (G1)	Neck dissection	1, 2, 2	
8	37	M	2/2	Squamous cell carcinoma (G1)	Oral cavity	1 (both)	Carcinoma immunoreactivity was 10% and 80%

anoctamins (28). The anoctamin proteins (also known as transmembrane protein 16) represent a novel family of membrane proteins with 10 members in mammals (29). The biological functions of anoctamins are presently poorly understood (18) and this has been the object of certain studies (28, 30-32). Several anoctamins are overexpressed in several cancer types and other diseases (30). As a consequence of different study groups involved in cell research that characterized this protein, several synonyms of DOG1 are known, *e.g.* anoctamin-1 (10, 11, 13), transmembrane member 16A (12, 16, 17, 31, 32), oral cancer overexpressed protein 2 (33), and tumour amplified and overexpressed sequence-2 (19, 34).

DOG1 is a specific Ca^{2+} -activated chloride channel (31, 35). The physiological activation of DOG1 appears to be triggered by cellular hypotonic swelling, facilitating cell-volume decrease (30). This capacity of DOG1 is clearly involved in the motility of cells (28-30). DOG1 is a valuable and specific histological marker of GIST (4). Beyond diagnostic applications in histological characterization of this rare gastrointestinal disease, DOG1 was found to be sporadically expressed in other tumour types, such as luminal ductal cells of salivary gland tumours (8), and was consequently proposed as a marker for salivary acinar and intercalated duct differentiation (9).

In cancer of the upper aerodigestive system, high expression levels of DOG1 correlated with poor survival in patients with oesophageal squamous cell carcinoma (30). DOG1 expression has been found to be associated with tumourigenesis and invasion of HNSCC (10-12). Recently,

Britschgi *et al.* confirmed findings of amplification and overexpression of this protein in several cancer types, including HNSCC (36). The authors revealed this protein to activate both epithelial growth factor receptor and calmodulin-dependent protein kinase II (36). These reactions lead to activation of protein kinase B/AK strain transforming and mitogen-activated protein kinase 1 signaling (36, 37). However, the immunohistochemical expression pattern of this protein differed between primary tumours and regional lymph node metastases of the same individual (n=21). Interestingly, the protein was expressed highly in primary cancer but only at low level in metastatic lymph nodes (14). This study group argued that the level of DOG1 expression indicates a switch between tumour growth and metastasis in HNSCC, with high levels in primary tumours being associated with local tumour progression and low levels in lymph nodes being a presumed prerequisite for metastasizing capabilities of carcinoma cells (14). These inverse relationships between the protein expression patterns in locally growing or mobile tumour cells were supported by *in vitro* investigations (13).

Another study reported a high number of DOG1-positive tumour cells (82.5%) in OSCC (13). In this extensive study, the immunoreactivity of a polyclonal antibody revealed a labelling of grade III differentiated OSCCs in 75% of cases (13). In fact, in their study, the differentiation of OSCC had no effect on antigen detection (13).

The aim of this study was to analyze the expression of DOG1 in routinely processed poorly/undifferentiated carcinoma of the upper aerodigestive tract.

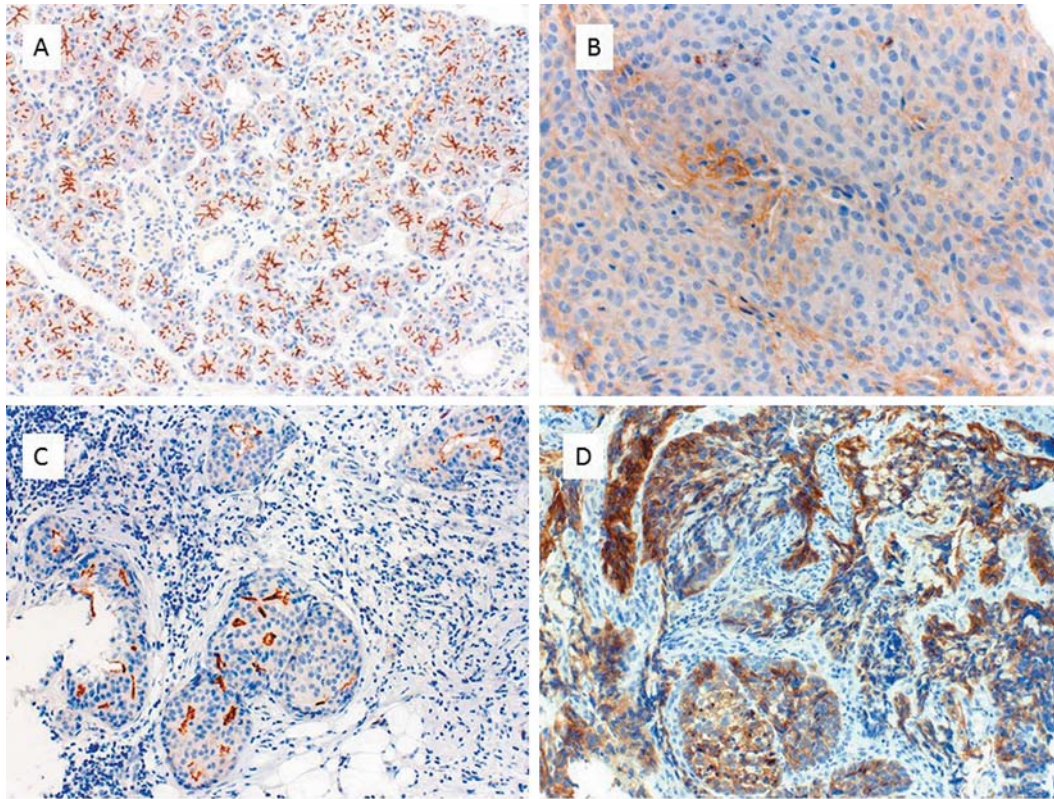


Figure 1. Immunoreactivity to discovered on gastrointestinal stromal tumour protein 1 (DOG1) antibody in normal salivary gland (A), lymphangiosis carcinomatosa (basal and parabasal tumour cells) (B), solid carcinoma, mandibular angle metastasis (basal tumour cells) (C), and solid nasopharyngeal carcinoma (all tumour cells) (D) (magnification: A, C, D $\times 50$, B: $\times 100$).

Materials and Methods

A total of 84 formalin-fixed, paraffin-embedded specimens from 31 patients with upper aerodigestive tract carcinoma were investigated for DOG1 expression (antibody to DOG1, clone SP31, dilution 1:100; Spring Bioscience Corp., Pleasanton, CA, USA). The avidin–biotin complex (ABC) reaction was used to visualize the binding of the primary antibody.

Immunoreactivity in carcinomas was estimated using a visual score (0: negative; 1: basally positive, 2: parabasally positive, 3: completely positive, 4: basally and parabasally positive).

Relevant studies were searched in PubMed/Medline database for combinations of terms “squamous cell carcinoma”, “salivary gland tumour/carcinoma”, “HNSCC”, “OSCC” and synonyms of “DOG1” to collate current studies on expression pattern of this protein in tumours of the upper aerodigestive tract.

Results

A total of 69 (82.1%) out of 84 specimen were negative for the DOG1 protein. DOG1-stained slices were restricted to eight patients (25.8%) (Table I). However, the staining pattern was not a regular finding in every specimen of each individual.

Completely stained samples were rare (n=3 patients, 9.6% of all individuals). Finally, staining was restricted to certain epithelial layers of carcinoma cells: basal (n=5), parabasal (n=5), completely (n=3), or basal/parabasal (n=2), respectively (Figure 1).

Results of a literature survey are presented in Table II.

Discussion

The present study demonstrated distinct patterns of expression of DOG1 in poorly differentiated carcinoma of the head and neck region. About 25% of patients with HNSCC expressed DOG1 in at least one specimen.

In clinical praxis, DOG1 immunoexpression is an important tool for diagnosing GIST (39, 40) and chondroblastoma (41). In the head and neck region, DOG1 is a useful marker for differentiating certain salivary gland neoplasms (7-9). Adenocarcinoma, such as esophageal and certain salivary gland carcinomas, also express DOG1. Using the K9 antibody to identify DOG1, Lopes *et al.* revealed DOG1 expression of carcinoma cells in three out of a total of

Table II. Selected publications detailing immunoreactivity for discovered on gastrointestinal tumour protein 1 (DOG1) in human upper aerodigestive tract tumours.

Author	Clone/Antibody	Entity/region	Number/percentage of positively stained samples	Remarks
Carles <i>et al.</i> 2006 (24)	Self-manufactured antibody	HNSCC	Not detailed	Characterization of DOG1 as potential therapeutic target
Miettinen <i>et al.</i> 2009 (3)	K9; Novocastra, Newcastle, UK	Oesophageal GIST	DOG1 positivity relatively common in oesophageal carcinoma	Identifies GIST of oesophagus
Ayoub <i>et al.</i> 2010 (10)	PAb2069	HNSCC (cell culture study)	83 Specimen on tissue array (HPV-negative)	Level of expression associated with local growth and metastasis
Kashyap <i>et al.</i> 2009 (33)	Oral cancer overexpressed 2 (ab53212); Abcam, Cambridge, UK	Oesophageal SCC (all grades)	85-98%; Expression differed between primaries and lymph node metastases	Valuable prognostic marker
Hemminger and Iwenofu 2012 (6)	K9, no. NCL-L-DOG1; Leica, Wetzlar	Salivary gland	<80% (Adenocarcinoma of different location immunoreactive to DOG1 antibody)	Recommended for AcicC differentiation
Ruiz <i>et al.</i> 2012 (11)	SP31; Cell Marque, Rocklin, CA, USA	HNSCC	Oral cavity: 5/103 (4.9%); Oropharynx: 3/68 (4.2%); Larynx 4/27 (12.9%); Hypopharynx: 7/37 (18.9%)	DOG1 immunoreactivity indicated poor survival
Chênevert J <i>et al.</i> 2012 (7)	1.1; Zeta, Sierra Madre, CA, USA	Salivary gland (acinar, intercalated duct, myoepithelial)	Acinar: 100%, diffuse: 93% ACC; 18/24 EMC 8/15	Also stained normal acinar cells
Jung <i>et al.</i> 2013 (9)	Rabbit polyclonal; Spring Science, Pleasanton, CA, USA	Salivary gland (MASC, mimic MASC, conventional AcicC)	MASC: 1/13 Mimic MASC: 0/10 AcicC: 3/6	Additional marker in diagnostics of MASC
Britschgi <i>et al.</i> 2013 (36)	SP31; Abcam	HNSCC (cell lines)	100% Inverse relation between DOG1 expression in primaries and that in lymph nodes	Valuable marker in HNSCC diagnosis
Montalli <i>et al.</i> 2014 (8)	Not detailed; Abcam	Salivary gland (basal cell adenoma)	Expression varied relative to cell types of salivary glands and salivary gland tumours	Expression in basal cell adenoma
Li <i>et al.</i> 2014 (13)	Anti-ANO1; Sigma, St. Louis, MO, USA	Oral mucosa: OSCC (160 patients)	Expression differed in primaries and lymph node metastases	Valuable prognostic marker
Pinto <i>et al.</i> 2014 (15)	Not detailed; Dako, Glostrup, Denmark	Salivary gland (MASC)	0 (Non-neoplastic acinar cells positive)	DOG1 staining useful for differential diagnosis from AcicC
Shiowski <i>et al.</i> 2014 (14)	SP31	HNSCC: carcinoma cells in primaries and lymph nodes (n=26)	100%: In 18/21 cases, DOG1 immunoreactivity stronger in primaries than in lymph nodes	Valuable marker in HNSCC diagnosis

ACC: Adenoid cystic carcinoma, AcicC: acinus cell carcinoma, EMC: epithelial myoepithelial carcinoma, HNSCC: head and neck squamous cell carcinoma, MASC: mammary analogue secretory carcinoma, HPV: human papilloma virus, GIST: gastrointestinal stromal tumour, OSCC: oral squamous cell carcinoma, SCC: squamous cell carcinoma.

four adenoid cystic carcinomas of the head and neck (4). Following recent studies on DOG1 expression in HNSCC, the demonstration of DOG1 expression in these entities is of significant prognostic relevance (11).

The present study provides data on only relatively rare and unpredictable expression of DOG1 in poorly differentiated carcinoma of the head and neck. On the other hand, the different designations given for the same protein illustrate the recognition of DOG1 in HNSCC (1, 10, 16, 19). In contrast to recent studies on OSCC (13, 14, 36) (Table II), a relatively small subset of HNSCCs from our study cohort exhibited DOG1 expression. However, this expression should be

evaluated with caution. In several cases, the DOG1 expression was analyzed in multiple samples of neck metastases or in different specimens from the same patient obtained during surgical treatment for recurrent disease. The expression pattern of DOG1 differed markedly, both in samples from different sites at the time of surgery and during repeated surgical intervention. It is likely that the heterogeneity of DOG1 expression illustrates one facet of the changing tumour biology of advanced-staged HNSCC.

Several studies have addressed the potential of DOG1 expression as a prognostic marker in HNSCC (13, 36). Some authors argue that a high level of DOG1 in malignant tumours

leads to enhanced cell motility, distant metastasis and poor prognosis (30, 36). Using a rabbit polyclonal antibody, Li *et al.* revealed a higher expression pattern of the antigen in metastatic than in non-metastatic in OSCC, and the staining intensity correlated with staging (13). This finding was also noted by Ruiz *et al.* (11). The number of DOG1-immunoreactive cases was low in carcinomas of several locations of the upper aerodigestive tract (11). On the other hand, one study reported a very high rate of DOG1-immunoreactive carcinoma cells in HNSCC (13). The differences between earlier studies on HNSCC and the present findings are only in part attributable to the use of different antibodies (Table II). Ruiz *et al.* applied the same DOG1 antibody in their study on DOG1 immunoreactivity of HNSCC as we used in the present investigation (11). The frequency of DOG1 immunoreactive HNSCC of the current study is similar to the study of Ruiz *et al.* (11). These authors observed a correlation of DOG1 expression in HNSCC with prognosis (11). However, we focused on the demonstration of DOG1 expression in a selected group of poorly differentiated HNSCC cases and did not address the outcome of our patients because it is unlikely that any prognostic status of DOG1 expression is possible in these severely affected individuals.

Conclusion

DOG1 is expressed in a subset of poorly differentiated HNSCC. The variable expression pattern of DOG1 in individual cases may possibly reflect the heterogeneity of advanced-stage tumours. The unpredictable expression of DOG1 in routinely processed HNSCCs does not allow any recommendation to be made for distinction of poorly differentiated carcinoma of this region using DOG1.

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