Dog1 Expression in Neuroendocrine Neoplasms: Potential Applications and Diagnostic Pitfalls

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Abstract

Neuroendocrine neoplasms (NENs) represent a heterogeneous group of rare tumors, more frequently arising from gastroenteropancreatic tract and lungs. At the time of diagnosis, 20% of cases are metastatic, and 10% of cases are considered as cancer of unknown primary origin. Several immunohistochemical markers are routinely used to confirm the neuroendocrine differentiation, first among all Synaptophysin and Chromogranin-A; on the other hand, different immunohistochemical markers are used to establish primary anatomical site, as TTF1, CDX2, Islet-1 and Calcitonin, but no marker is available in order to distinguish among different sites of the digestive tract.

DOG1 (discovered on GIST-1) is a gene normally expressed in interstitial cells of Cajal and, in routine practice, DOG1 immunostaining is used in diagnosis of GIST (gastrointestinal stromal tumor). DOG1 expression has been described in several neoplasms other than GIST, both in mesenchymal and epithelial neoplasms. In the present study, DOG1 immunostaining has been performed in a large cohort of neuroendocrine neoplasms, including neuroendocrine tumors and neuroendocrine carcinomas, in order to evaluate frequency, intensity and pattern of expression in different anatomical site and in different tumor grade. DOG1 expression was detected in a large percentage of neuroendocrine tumors, with statistically significant association between DOG1 expression and gastrointestinal tract neuroendocrine tumors. As a consequence, DOG1 could be included in marker panel for the identification of primary site in neuroendocrine metastases of unknown primary origin; moreover, these results recommend careful evaluation of DOG1 expression in gastrointestinal neoplasms, in particular in differential diagnosis between epithelioid GIST and neuroendocrine tumors.

Introduction

Neuroendocrine neoplasms (NENs) are a heterogeneous group of rare tumors, accounting for 2% of all malignancies, with an increasing incidence of 2–5 cases per 100000 per year, with common primary sites represented by digestive tract, pancreas and lung[1–4]. Metastases are found at the time of diagnosis in 20–22% of patients, more frequently to the liver, and in 10% of patients the primary site remains unknown[5, 6]. Considering that recommended therapies are tailored for different primary site, identifying anatomical origin is increasingly important for clinical management[7]. In routine practice, several well known immunomarkers are available for defining neuroendocrine differentiation, as Synaptophysin, Chromogranin-A, CD56 and NSE (neuron specific enolase); on the other side, immunomarkers for establishing primary site include TTF1 for lung, CDX2 for gastrointestinal tract, ISL1 for pancreas, Calcitonin for medullary thyroid carcinoma and polyomavirus in Merkel cell carcinoma. However, currently no immunohistochemical panel allows reliable identification of precise anatomical site among the digestive tract[7, 8].

On the other side, DOG-1 (discovered on GIST-1) is a gene that encodes a Calcium-activated chloride channel, also known as Anoctamin1 (ANO1) or TMEM16A, physiologically expressed in interstitial cells of Cajal[9–12]. In routine practice, DOG1 antibody is used as immunohistochemical marker in diagnosis of
gastrointestinal stromal tumor (GIST)[13, 14], with high sensitivity (ranging between 87% and 94.4% depending on the antibody clone), and high specificity[15–17]. In literature, several studies evaluated DOG1 expression in both mesenchimal and epithelial neoplasms other than GIST; indeed, among mesenchimal neoplasms, DOG1 reactivity has been found in low-grade fibromyxoid sarcoma, gastrointestinal leiomyoma, myxofibrosarcoma, synovial sarcoma, myxoid liposarcoma, undifferentiated pleomorphic sarcoma and in a prostatic stromal sarcoma[18–22]; as concerns epithelial neoplasms, DOG1 expression has been proved in cases of salivary gland, colorectal and endometrial adenocarcinomas, in pancreatic adenocarcinoma and in pancreatic solid pseudopapillary neoplasm, in testicular tumors and in some histotypes of renal cell carcinoma, in poorly differentiated head and neck carcinoma and in a subset of adnexal tumors, with particular correlation with eccrine differentiation neoplasm[16, 23–30]. Furthermore, DOG1 has also been evaluated as a prognostic marker in breast cancer, with conflicting results[31, 32]. Furthermore, DOG1 expression has also been studied as a neuroendocrine marker in the spectrum of non-neoplastic neuroendocrine cells and in neuroendocrine neoplasms, with a few cases of neuroendocrine carcinomas which stained positive for DOG1 antibody[32–35]; however, in literature, no studies with focus on DOG1 expression in gastrointestinal NENs are currently available.

The aim of this study was to perform DOG1 immunostaining on a large cohort of neuroendocrine neoplasms in order to provide a systematic evaluation of frequency, intensity and expression pattern in different primary sites and different grades neuroendocrine neoplasms.

**Materials And Methods**

**Samples**

A total of 197 consecutive cases of neuroendocrine neoplasms (NENs) surgically resected or biopsied from 2015 to 2021 were retrieved from Pathology Department in Niguarda Hospital, Milan, Italy. All cases were reviewed in order to confirm the diagnosis; tumor grading was defined according to the latest WHO guidelines available at the time of the study. We collected 131 neuroendocrine tumors (NETs), 40 neuroendocrine carcinomas (NECs), 25 medullary carcinomas of thyroid (MTC) and one Merkel cell carcinoma (MCC). Among NETs, 116 well-differentiated (G1) NETs (including 34 pulmonary typical carcinoids, TC), 14 moderately differentiated (G2) NETs (including 3 pulmonary atypical carcinoids, AC), and 1 poorly-differentiated (G3) NET were included. The cases were further subdivided according to the primary tumor site.

**Immunohistochemical analyses and DOG1 assessment**

A representative formalin-fixed paraffin-embedded (FFPE) tissue block for each case was obtained from our archives, and immunohistochemical analyses were performed on 3 µm thick sections on polarized glass slides using an automated system (Dako Omnis; Agilent) and the following antibodies: cytokeratin cocktail (clone AE1/AE3, ready to use dilution; Dako/Agilent), Synaptophysin (ready to use dilution; Dako/Agilent), Chromogranin-A (dilution used 1:1000; Dako/Agilent), Ki67 (clone MIB-1, ready to use
dilution; Dako/Agilent) and DOG1 (clone SP31; dilution used 1:50; GENNOVA). Immunohistochemical analyses were evaluated by two surgical pathologists and a resident fellow.

For each DOG1 immunostaining, percentage and distribution of positive neoplastic cells (diffuse or focal) and staining pattern (membranous and/or cytoplasmatic) were defined; next, staining intensity was scored with a semi-quantitative system, as faint (score 1+), moderate (score 2+) or intense (score 3+). Moderate or intense staining in more than 5% of tumor cells was considered positive.

**Statistical analyses**

Association of DOG1 positivity to the variables examined (primary tumor site and differentiation grade) was evaluated with Fisher's Exact test or Pearson's Chi-squared test, depending on the number of variables under consideration. Among neuroendocrine carcinomas, primary tumor site with two or less cases were excluded from statistical analyses. A p-value less or equal to 0.05 was considered statistically significant.

**Results**

A total of 197 cases of neuroendocrine neoplasms were evaluated, including 131 neuroendocrine tumors, 40 neuroendocrine carcinomas, 25 medullary thyroid carcinomas and 1 Merkel cell carcinoma.

Considering every intensity staining (1+, 2+ and 3+), DOG1 was expressed in 73/131 (55.7%) NETs, in 16/40 (40.0%) NECs and in 3/25 (12.0%) MTCs, while the single MCC case was completely negative for DOG1 (data not shown). In the evaluation of the association with differentiation grade, DOG1 expression was more common in NETs G2 (60/116, 51.7%) than in NETs G1 (12/14, 85.7%), with statistically significant difference (p-value = 0.021). In the single case of NET G3 of colorectal origin, DOG1 was strongly expressed (3+) in 98% of the tumor cells. Evaluating association of immunostaining with different primary tumor sites, DOG1 was positive in in 22/22 (100%) gastric NETs, in 9/10 (90.0%) duodenal NETs, in 1/3 (33.3%) NETs of the papilla of Vater, in 19/20 (95.0%) ileal NETs, in 2/2 (100%) NETs of the ileal-cecal valve, in 4/5 (80.0%) appendiceal NETs, in 3/4 (75.0%) NETs of colorectum, in 6/28 (21.4%) pancreatic NETs, and in 7/37 (18.9%) typical and atypical pulmonary carcinoids. Among neuroendocrine carcinomas, DOG1 positivity was found in 6/22 (27.2%) pulmonary NECs, in 1/2 (50.0%) gastric NECs, in 2/3 (66.7%) duodenal NECs, in 1/1 (100%) NEC of the papilla of Vater, in 2/4 (50.0%) colorectal NECs, in 1/2 (50.0%) prostatic carcinomas, in 2/2 (100%) NECs of the urinary bladder and in 1/1 (100%) carcinoma of the pharynx. The single cases of neuroendocrine carcinomas of gallbladder, breast and paranasal sinus were negative for DOG1 immunostaining.

Considering DOG1 staining as positive if 5% or more of tumor cells demonstrate moderate (2+) or strong (3+) intensity staining, a total of 56/131 (42.7%) NETs, 12/40 (30.0%) NECs and 1/25 (4.0%) MTC resulted positive (Table 1). As specified in "Materials and Methods", primary sites with 2 or less cases were not included in statistical analysis. As concerns differentiation grade, 46/116 (39.7%) NETs G1, 9/14 (64.3%) NETs G2 and 1/1 (100%) NETs G3 were positive, without statistical differences between G1 and
G2 neuroendocrine tumors (p-value = 0.092). Evaluating DOG1 expression in different primary tumor sites, DOG1 positivity was identified in 19/22 (86.4%) gastric NETs, in 6/10 (60.0%) duodenal NETs, in 1/3 (33.3%) NET of the papilla of Vater, in 17/20 (85.0%) ileal NETs, in 1/2 (50.0%) NET of the ileal-cecal valve, in 3/5 (60.0%) NETs of the appendix, in 3/4 (75.0%) colorectal NETs, in 3/28 (10.7%) pancreatic NETs and in 3/37 (8.1%) pulmonary carcinoid. Interestingly, DOG1 expression was statistically more frequent (p-value < 0.001) in digestive tract neuroendocrine tumors (50/66, 75.6%) compared to pulmonary carcinoids (3/37, 8.1%) and pancreatic NETs (3/28, 10.1%) (Table 2). Taking into account both the differentiation grade and the site of origin, DOG1 was found positive in in 17/19 (89.5%) NETs G1 and 2/3 (66.7%) NET G2 of the stomach, in 13/16 (81.2%) NETs G1 and 4/4 (100%) NETs G2 of ileum, in 2/3 (66.7%) NET G1 and 1/1 (100%) NET G3 of the colorectum, in 2/24 (8.3%) NET G1 and 1/4 (25.0%) NET G2 of the pancreas, and in 1/34 (2.9%) TCs and 2/3 (66.7%) ACs of the lung. DOG1 positivity in NECs was identified in 1/2 (50.0%) gastric carcinomas, in 1/3 (33.3%) duodenal NEC, in 1/1 (100%) NEC of the papilla of Vater, in 1/4 (25.0%) colorectal NEC, in 5/22 (22.7%) lung carcinomas, in 2/2 (100%) NECs of the urinary bladder and in 1/1 (100%) NEC of the pharynx, while the NECs of gallbladder, breast, prostate and paranasal sinus were completely negative. So, also between NECs, digestive tract tumors were more frequently associated with DOG1 immunoreactivity (4/10, 40.0%), compared to pulmonary NECs (5/22, 22.3%), but the association was not statistically significant (p-value = 0.45) (Table 2). Only one case of MC of the thyroid out of 25 (4.0%) was classified as positive with sufficient intensity (2+ or 3+) and tumor cell percentage (> 5%) expression.
Table 1
Frequency of DOG1 positivity, divided for anatomical site and differentiation grade, with staining considered as positive if 5% or more of tumor cells demonstrate moderate (2+) or strong (3+) intensity staining.

<table>
<thead>
<tr>
<th>Primary tumor site</th>
<th>NET G1 and TC</th>
<th>NET G2 and AT</th>
<th>NET G3</th>
<th>Total of NET</th>
<th>NEC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stomach</td>
<td>17/19 (89.5%)</td>
<td>2/3 (66.7%)</td>
<td>-</td>
<td>19/22 (86.3%)</td>
<td>1/2 (50.0%)</td>
</tr>
<tr>
<td>Duodenum</td>
<td>6/10 (60.0%)</td>
<td>-</td>
<td>-</td>
<td>6/10 (60.0%)</td>
<td>1/3 (33.3%)</td>
</tr>
<tr>
<td>Ileum</td>
<td>13/16 (81.3%)</td>
<td>4/4 (100%)</td>
<td>-</td>
<td>17/20 (85.0%)</td>
<td>-</td>
</tr>
<tr>
<td>Ileocecal valve</td>
<td>1/2 (50.0%)</td>
<td>-</td>
<td>-</td>
<td>1/2 (50.0%)</td>
<td>-</td>
</tr>
<tr>
<td>3/5 (60.0%)</td>
<td>-</td>
<td>-</td>
<td>3/5</td>
<td>(60.0%)</td>
<td>-</td>
</tr>
<tr>
<td>Colorectum</td>
<td>2/3 (66.7%)</td>
<td>-</td>
<td>1/1</td>
<td>(100%)</td>
<td>1/4 (25.0%)</td>
</tr>
<tr>
<td>Papilla of Vater</td>
<td>1/3 (33.3%)</td>
<td>-</td>
<td>-</td>
<td>1/3 (33.3%)</td>
<td>1/1 (100%)</td>
</tr>
<tr>
<td>Pancreas</td>
<td>2/24 (8.3%)</td>
<td>1/4 (25.0%)</td>
<td>-</td>
<td>3/28 (10.7%)</td>
<td>-</td>
</tr>
<tr>
<td>Gallbladder</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0/1 (0%)</td>
</tr>
<tr>
<td>Bladder</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>2/2 (100%)</td>
</tr>
<tr>
<td>Prostate</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0/2 (0%)</td>
</tr>
<tr>
<td>Breast</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0/1 (0%)</td>
</tr>
<tr>
<td>Paranasal Sinuses</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0/1 (0%)</td>
</tr>
<tr>
<td>Pharynx</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1/1 (100%)</td>
</tr>
<tr>
<td>Lung</td>
<td>1/34 (2.9%)</td>
<td>2/3 (66.7%)</td>
<td>3/37</td>
<td>(8.1%)</td>
<td>5/22 (22.7%)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>46/116</strong> (39.7%)</td>
<td><strong>9/14</strong> (64.3%)</td>
<td><strong>1/1</strong> (100%)</td>
<td><strong>56/131</strong> (42.7%)</td>
<td><strong>12/40</strong> (30.0%)</td>
</tr>
<tr>
<td>Thyroid</td>
<td></td>
<td></td>
<td></td>
<td>1/25 (4.0%)</td>
<td></td>
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<tr>
<td>Skin</td>
<td></td>
<td></td>
<td></td>
<td>0/1 (0%)</td>
<td></td>
</tr>
</tbody>
</table>

NET: neuroendocrine tumor

NEC: neuroendocrine carcinoma

TC: pulmonary typical carcinoid

AC: pulmonary atypical carcinoid
Collaterally, DOG1 expression in nonneoplastic cells was evaluated, with interesting findings. Endocrine cells of gastric and ileal mucosa, identified with both morphology and Synaptophysin and Chromogranin-A, also expressed DOG1 (Fig. 1G), a peculiar finding even more evident in neuroendocrine cell hyperplasia in the setting of autoimmune atrophic gastritis. Conversely, we couldn't identify any DOG1 expression in pancreatic endocrine cells, in contrast with results described by Ardeleanu’s[33].

**Discussion**

DOG1 is a sensitive and specific immunomarker for GIST[13–15], but its expression is also well studied in many different non-GIST neoplasms, especially in several epithelial tumors; however, DOG1 expression in NENs has been reported but not systematically evaluated.

In this study, DOG1 immunohistochemical staining was evaluated in a total of 197 neuroendocrine neoplasms with different primary site and differentiation grade, and neuroendocrine tumors of digestive tract resulted to be more frequently positive than NETs of other primary sites, with statistically significance. Otherwise, DOG1 in NECs was less commonly expressed, and no significant difference was found between different anatomical sites. These results differ from those of the study carried out by Jansen et al[32], where only one cases of pancreatic neuroendocrine neoplasms shown positivity to DOG1 immunostaining, among a large cohort of NENs. Possible explanations of this difference are the tumor heterogeneity, which could have result in negative staining, since Jansen et al used 0,6 mm tissue cores microarrays.

As concerns nonneoplastic tissue, in the present study only endocrine cells of gastric and ileal mucosa expressed DOG1, while pancreatic and bronchial endocrine cells were constantly negative, in contrast to Ardeleanu’s findings[33]. A possible explanation could be the different specificity of the DOG1 clone, since Ardeleanu ed al used a polyclonal anti-DOG1 antibody as compared to our monoclonal antibody. Based on these findings, DOG1 seems to be not an endocrine marker, as previously proposed[33], but more probably it could mark only endocrine cell originating from the digestive tract.

In conclusion, our data show a strong correlation between DOG1 expression neuroendocrine tumors arising in the digestive tract. In light of these findings, DOG1 expression could be used as part of immunohistochemical panel for the purpose of defining primary anatomical site in the challenging setting of NET of unknown origin, suggesting clinical and radiological investigation of digestive tract. On the other side, our results highlight the need of careful evaluation and interpretation of DOG1 expression in the differential diagnosis between NET and GIST, particularly with epithelioid morphology, where association of other specific markers is recommended to avoid misdiagnosis, especially in small biopsies or in cytological specimens.

**Declarations**

Ethical Approval
This declaration is not applicable.

Competing interests

We have no competing interests

Authors' contributions

All listed authors contributed to the production of this manuscript and are listed in the appropriate order. All authors reviewed the manuscript.

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Availability of data and materials

Our datasets used can be accessed

References


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Tables

Tables 2 is available in the Supplementary Files section.

Figures
Figure 1

DOG1 immunostaining. A) Gastric NET is negative, while mucosal neuroendocrine cell are positive; B) Pancreatic NET with weak cytoplasmic staining; C) Ileal NET shows moderate (2+) positivity, with membranous and cytoplasmic pattern; D) Strong (3+) and diffuse positivity in gastric NET; E) NEC of the lung completely negative; F) Colic NEC with moderate (2+) staining; G) Normal ileal mucosa, with
scattered neuroendocrine cells positive for DOG1; Gastric mucosa with autoimmune atrophic gastritis and hyperplastic neuroendocrine cells staining for DOG1.

**Supplementary Files**

This is a list of supplementary files associated with this preprint. Click to download.

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