Synaptophysin and chromogranin A expression analysis in human tumors

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ABSTRACT

The expression of the neuroendocrine markers synaptophysin and chromogranin A was analyzed by immunohistochemistry in 14,584 samples from 103 different tumor types and subtypes in a tissue microarray format. At least one of these markers was found to be positive in 96.7% of tumors from various subtypes of neuroendocrine neoplasms. In non-neuroendocrine tumors, synaptophysin and/or chromogranin A staining was seen in 6.3% (n = 584), specifically in 41 of 88 non-neuroendocrine tumor entities. Basal cell carcinomas of the skin (50% positive for chromogranin A alone) and adenocortical carcinomas (91.7% positive for synaptophysin alone) stood out due to a frequent expression of only one specific marker. A subdivision of non-neuroendocrine neoplasms revealed “neuroendocrine differentiation” most commonly in adenocarcinomas from the female genital tract (18.9%), from pancreatico-/hepato-/biliary tract (15.8%) and the prostate (14.9%) while it was rare in urothelial (1.0%) and squamous cell carcinomas (0.6%). A comparison with clinico-pathological parameters of tumor aggressiveness did not suggest a clinical significance of neuroendocrine marker expression in 204 endometrium cancers, 249 pancreatic adenocarcinomas, 233 gastric adenocarcinomas and 1,182 colorectal adenocarcinomas. Within a cohort of 1,073 breast cancers of no special type, synaptophysin positivity was seen in 4.9% of cases and it was significantly linked to advanced tumor stage (p = 0.0427), high tumor grade (p = 0.0319) and loss of estrogen receptor expression (p = 0.0061) but unrelated to patient outcome. In conclusion, “neuroendocrine differentiation” can be observed in many different tumor types with non-neuroendocrine morphology. Evidence for a statistically significant association (p < 0.0001) between such a “neuroendocrine differentiation” and tumor aggressiveness could not be found.

1. Introduction

Neuroendocrine differentiation can be defined by its (patho-)physiological property of secretion of bioactive substances into the bloodstream or pathologically by distinct architectural and cytological patterns as well as immunohistochemically through detection of characteristic neurosecretory proteins such as synaptophysin and chromogranin A. Synaptophysin is the most abundant synaptic vesicle protein by mass (Takamori et al., 2006) and regulates endocytosis of synaptic vesicles (Kwon and Chapman, 2011). Synaptophysin is a commonly used immunohistochemical marker with a high sensitivity and a limited specificity, especially in high grade tumors (Rindi et al., 2022). The second most commonly used immunohistochemical marker for determining neuroendocrine differentiation is chromogranin A, which is highly specific but less sensitive than synaptophysin (Rindi et al., 2022). Chromogranin A is a granin protein located in secretory vesicles of neurons and endocrine cells (Bartolomucci et al., 2011) and is involved in various biological pathways controlling protein (peptides, hormones, neurotransmitters and growth factors) secretion upon stimulation (Arvan et al., 1991). In recent years, INSM1 (Insulinoma-associated...
protein) has increasingly been proposed as an (additional) nuclear marker for neuroendocrine differentiation in diagnostic pathology. Further studies are needed to confirm the diagnostic utility as a stand-alone or additional marker for identifying neuroendocrine differentiation.

Expression of neuroendocrine features is an important hallmark of neuroendocrine neoplasms. Tumors with neuroendocrine features can be basically divided into four main groups: 1.) well differentiated neuroendocrine tumors, 2.) poorly differentiated neuroendocrine carcinomas, 3.) tumors with neuroendocrine and non-neuroendocrine components (MiNEN, mixed neuroendocrine/non-neuroendocrine neoplasm), and 4) tumors with non-neuroendocrine morphology but expression of neuroendocrine markers (reviewed in (Volante et al., 2006)). Terminology and classification systems vary by organ and have changed over time, although this basic division applies to most organs reviewed in (Rindi et al., 2022).

The clinical significance of tumors of the groups 1–3 has been extensively studied and is considerably well known for neoplasms derived from most organs. The clinical significance of neuroendocrine marker expression in non-neuroendocrine appearing tumors is much less clear, however. Proposals for using “neuroendocrine marker expression” for classifying tumors of non-neuroendocrine origin exist for some tumor entities such as breast and prostate cancer but are lacking for many other tumor entities (2019,2016). Moreover, the incidence of carcinomas showing only neuroendocrine differentiation by immunohistochemistry (group 4 mentioned above) is probably underestimated, since immunostaining for neuroendocrine markers is not performed routinely.

To better understand the prevalence of “neuroendocrine differentiation” in cancer, a comprehensive study analyzing a large number of cancer tissues under highly standardized conditions is needed. Therefore, synaptophysin and chromogranin A were analyzed in more than 14,584 tumor tissue samples from 103 different tumor types and subtypes as well as 76 non-neoplastic tissue categories by immunohistochemistry (IHC) in a tissue microarray format in this study.

2. Material and methods

Tissue Microarrays (TMAs). The normal tissue TMA was composed of 8 samples from 8 different donors for each of 76 different normal tissue types (608 samples on one slide). The cancer TMAs contained a total of 14,584 primary tumors from 103 tumor types and subtypes. Detailed histopathological data on grade, pT and pN status (plus estrogen receptor, progesteron receptor and HER2 status for breast cancer) were available from 259 endometrial, 2,139 breast, 598 pancreatic, 327 gastric and 2,351 colorectal cancers. Clinical follow-up data were only available from 877 breast cancer patients. In these patients the median follow-up time was 43 (range 1–88) months. The composition of both normal and tumor TMAs is described in detail in the results section. All samples were retrieved from the archives of the Institutes of Pathology, University Hospital of Hamburg, Germany, the Institute of Pathology, Clinical Center Osnabrueck, Germany, and Department of Pathology, Academic Hospital Fuert, Germany. Tissues were fixed in 4% buffered formalin and then embedded in paraffin. The TMA manufacturing process was described earlier in detail (Dancau et al., 2016; Kononen et al., 1998). In brief, one tissue spot (diameter: 0.6 mm) was transmitted from a tumor containing donor block in an empty recipient paraffin block. The use 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. The TMAs have been used in previous studies (Uhlig et al., 2022; Reischwig et al., 2021; Menz et al., 2021), although the total number of analyzed tumors varies between different studies since a combination of different TMA blocks is sometimes used.

Immunohistochemistry (IHC). Freshly prepared TMA sections were manually 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 min in an autoclave at 121 °C in pH 7.8 buffer. Endogenous peroxidase activity was blocked with Dako Peroxidase Blocking Solution™ (Agilent, CA, USA; #52023) for 10 min. Primary antibody specific against synaptophysin (rabbit recombinant, MSVA-462R, MS Validated Antibodies GmbH, Hamburg, Germany) was applied at 37 °C for 60 min at a dilution of 1:2000 and against chromogranin A (rabbit recombinant, MSVA-380R, MS Validated Antibodies) was applied at 37 °C for 60 min at a dilution of 1:3600. Bound antibody was then visualized using the EnVision Kit™ (Agilent, CA, USA; #K5007) according to the manufacturer’s directions. The sections were counterstained with haemalum (Dako, EnVision Flex, SM806, Agilent, CA, USA). For tumor tissues, the percentage of marker positive tumor cells was estimated and the staining intensity was semi-quantitatively recorded (0, 1+, 2+, 3+). For statistical analyses, the staining results were categorized into four groups as follows (see also Suppl. Table 1): Negative: no staining at all, weak staining: staining intensity of 1+ in ≤70% or staining intensity of 2+ in ≤30% of tumor cells, moderate staining: staining intensity of 1+ in >70%, staining intensity of 2+ in >30% but in ≤70% or staining intensity of 3+ in ≤30% of tumor cells, strong staining: staining intensity of 2+ in >70% or staining intensity of 3+ in >30% of tumor cells.

Statistics. Statistical calculations were performed with JMP 14 software (SAS Institute Inc., NC, USA). Contingency tables and the chi² test were performed to search for associations between synaptophysin/ chromogranin A and tumor phenotype. Survival curves were calculated according to Kaplan-Meier. The Log-Rank test was applied to detect significant differences between groups. A p-value of ≤0.05 was defined as significant.

3. Results

Technical issues. The stainings were interpretable in 13,405 (91.9%) tumors for synaptophysin and in 11,218 (76.9%) for chromogranin A. A total of 9,697 (66.5%) samples had an interpretable result for both markers and were available for a combined analysis. Non-interpretable samples were due to lack of unequivocal tumor cells or loss of the tissue spot during technical procedures. A sufficient number of samples of each normal tissue type was always evaluable.

Synaptophysin immunostaining in normal tissues. Synaptophysin was strongly expressed in cerebrum, cerebellum, islets of Langerhans of the pancreas, the medulla of the adrenal gland, the anterior and posterior lobe of the pituitary gland, and in scattered cells of the diffuse neuroendocrine system, which are especially seen in gastrointestinal (including Brunner glands), respiratory, and endocervical epithelium as well as in the skin. A weak to strong immunostaining occurred in axons and ganglion cells of the peripheral nerves in the gastrointestinal wall. Some synaptophysin staining was also seen in adenral cortical cells, goblet cells and Paneth cells. Images of synaptophysin staining in normal tissues are shown in Fig. 1A–D.

Chromogranin A immunostaining in normal tissues. A strong Chromogranin A immunostaining was seen in the medulla but not in the cortex of the adrenal gland, the parathyroid, the anterior lobe of the pituitary gland, pancreatic islets of Langerhans, as well as in scattered cells of the diffuse neuroendocrine system. A weak to moderate immunostaining was also seen in axons and ganglion cells of the peripheral nerves in the gastrointestinal wall. Images of chromogranin A staining in normal tissues are shown in Fig. 1E–H.

Synaptophysin and Chromogranin A immunostaining in neoplastic tissues. A positive immunostaining was seen for synaptophysin in 6.5% of 13,405 cases (180 weak, 150 moderate, 544 strong; Supplementary Table 2) and for chromogranin A in 7.6% of 11,218 cases (220 weak, 273 moderate, 359 strong; Supplementary Table 3). 500 tumors (5.2%) were positive for both markers, 247 (2.5%) for synaptophysin only and 282 (2.9%) for chromogranin A only. Both markers

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Table 1
Synaptophysin and chromogranin A staining in tumors. (Weak, moderate and strong staining is summarized as positive staining. The neuroendocrine neoplasms are highlighted in grey). Note: In this table only tumors were included which had an interpretable result for synaptophysin and chromogranin A.

| Tumor entity                                      | Analyzable (n) | CGA negative / SYN negative (n,%) | CGA negative / SYN positive (n,%) | CGA positive / SYN negative (n,%) | CGA positive / SYN positive (n,%) |
|---------------------------------------------------|----------------|-----------------------------------|-----------------------------------|-----------------------------------|-----------------------------------|
| **Tumors of the skin**                            |                |                                   |                                   |                                   |                                   |
| Pilomatricoma                                     | 38             | 33,100                            | 0,0                               | 0,0                               | 0,0                               |
| Basal cell carcinoma                              | 88             | 32,50                             | 0,0                               | 32,50                             | 0,0                               |
| Benign nevus                                      | 29             | 26,100                            | 0,0                               | 0,0                               | 0,0                               |
| Squamous cell carcinoma of the skin               | 90             | 76,974                            | 1,13                              | 1,13                              | 0,0                               |
| Malignant melanoma                                | 46             | 43,100                            | 0,0                               | 0,0                               | 0,0                               |
| **Tumors of the head and neck**                   |                |                                   |                                   |                                   |                                   |
| Merkel cell carcinoma                             | 46             | 0,0                               | 8,182                             | 1,23                              | 35,795                            |
| Squamous cell carcinoma of the larynx             | 110            | 48,100                            | 0,0                               | 0,0                               | 0,0                               |
| Oral squamous cell carcinoma (floor of the mouth) | 130            | 49,100                            | 0,0                               | 0,0                               | 0,0                               |
| Basal cell adenoma of the salivary gland          | 15             | 15,100                            | 0,0                               | 0,0                               | 0,0                               |
| **Tumors of the lung, pleura and thymus**         |                |                                   |                                   |                                   |                                   |
| Adenocarcinoma of the lung                        | 196            | 161,97                            | 4,24                              | 0,0                               | 1,06                              |
| Squamous cell carcinoma of the lung               | 80             | 64,100                            | 0,0                               | 0,0                               | 0,0                               |
| Small cell carcinoma of the lung                  | 15             | 2,143                             | 3,214                             | 2,143                             | 7,50                              |
| Mesothelioma, epithelioid                         | 39             | 19,100                            | 0,0                               | 0,0                               | 0,0                               |
| Mesothelioma, other types                         | 76             | 52,981                            | 1,19                              | 0,0                               | 0,0                               |
| Thymoma                                           | 29             | 28,100                            | 0,0                               | 0,0                               | 0,0                               |
| **Tumors of the female genital tract**            |                |                                   |                                   |                                   |                                   |
| Squamous cell carcinoma of the vagina             | 78             | 35,100                            | 0,0                               | 0,0                               | 0,0                               |
| Squamous cell carcinoma of the vulva              | 130            | 39,100                            | 0,0                               | 0,0                               | 0,0                               |
| Squamous cell carcinoma of the cervix             | 129            | 43,100                            | 0,0                               | 0,0                               | 0,0                               |
| Endometrioid endometrial carcinoma                | 236            | 122,635                           | 3,16                              | 63,328                            | 4,21                              |
| Endometrial serous carcinoma                      | 82             | 61,91                             | 1,15                              | 5,75                              | 0,0                               |
| Carcinosarcoma of the uterus                      | 48             | 30,857                            | 2,57                              | 2,57                              | 1,29                              |
| Endometrioid carcinoma, high grade, G3            | 13             | 8,727                             | 2,182                             | 1,91                              | 0,0                               |
| Endometrioid clear cell carcinoma                 | 8              | 5,100                             | 0,0                               | 0,0                               | 0,0                               |
| Endometrioid carcinoma of the ovary               | 110            | 65,844                            | 3,39                              | 9,117                             | 0,0                               |
| Serous carcinoma of the ovary                     | 559            | 281,93                            | 1,03                              | 16,53                             | 4,13                              |
| Mucinous carcinoma of the ovary                   | 96             | 39,902                            | 2,31                              | 11,169                            | 13,20                             |
| Clear cell carcinoma of the ovary                 | 50             | 29,906                            | 0,0                               | 3,94                              | 0,0                               |
| Carcinosarcoma of the ovary                       | 47             | 32,941                            | 1,29                              | 1,29                              | 0,0                               |
| Brenner tumor                                     | 9              | 9,100                             | 0,0                               | 0,0                               | 0,0                               |
| Tumor entity                                                                 | analysisable (n) | CGA negative / SYN negative (n,%): | CGA positive / SYN positive (n,%): |
|------------------------------------------------------------------------------|------------------|-----------------------------------|-----------------------------------|
| **Tumors of the breast**                                                      |                  |                                   |                                   |
| Invasive breast carcinoma of no special type                                  | 1345             | 1048;94.1                         | 13;12                            |
| Lobular carcinoma of the breast                                              | 293              | 181;95.3                          | 1;0.5                            |
| Medullary carcinoma of the breast                                            | 26               | 25;100                            | 0;0                              |
| Tubular carcinoma of the breast                                              | 27               | 21;95.5                           | 1;4.5                            |
| Mucinous carcinoma of the breast                                             | 58               | 29;74.4                           | 2;5.1                            |
| Phyllodes tumor of the breast                                                | 50               | 47;100                            | 0;0                              |
| **Tumors of the digestive system**                                           |                  |                                   |                                   |
| Adenomatous polypl, low-grade dysplasia                                       | 50               | 47;100                            | 0;0                              |
| Adenomatous polypl, high-grade dysplasia                                      | 50               | 47;100                            | 0;0                              |
| Adenocarcinoma of the colon                                                  | 1882             | 1164;93.3                         | 34;2.7                           |
| Gastric adenocarcinoma, diffuse type                                         | 176              | 89;89                             | 6;6                              |
| Gastric adenocarcinoma, intestinal type                                       | 174              | 131;94.9                          | 2;1.0                            |
| Gastric adenocarcinoma, mixed type                                           | 62               | 37;97.4                           | 1;2.6                            |
| Adenocarcinoma of the esophagus                                              | 83               | 62;88.6                           | 3;4.3                            |
| Squamous cell carcinoma of the esophagus                                     | 75               | 66;100                            | 0;0                              |
| Squamous cell carcinoma of the anal canal                                    | 89               | 45;100                            | 0;0                              |
| Cholangiocarcinoma                                                           | 113              | 74;100                            | 0;0                              |
| Hepatocellular carcinoma                                                     | 50               | 49;100                            | 0;0                              |
| Ductal adenocarcinoma of the pancreas                                        | 612              | 250;82                            | 24;7.9                           |
| Pancreatic/Ampullary adenocarcinoma                                          | 89               | 39;78                             | 4;8                              |
| Acinar cell carcinoma of the pancrean                                        | 16               | 9;60                              | 3;2.0                            |
| Gastrointestinal stromal tumor (GIST)                                        | 50               | 48;98                             | 1;2                               |
| **Tumors of the urinary system**                                             |                  |                                   |                                   |
| Non-invasive papillary urothelial carcinoma, pTa G2 low-grade                | 177              | 94;100                            | 0;0                              |
| Non-invasive papillary urothelial carcinoma, pTa G2 high grade               | 141              | 66;100                            | 0;0                              |
| Non-invasive papillary urothelial carcinoma, pTa G3                          | 187              | 67;98.5                           | 1;1.5                            |
| Urothelial carcinoma, pT2-4 G3                                               | 1206             | 490;99                            | 2;0.4                            |
| Small cell neuroendocrine carcinoma of the bladder                           | 20               | 1;5.3                             | 9;47.4                           |
| Sarcomatoid urothelial carcinoma                                             | 25               | 8;100                             | 0;0                              |
| Clear cell renal cell carcinoma                                              | 857              | 629;100                           | 0;0                              |
| Papillary renal cell carcinoma                                               | 255              | 166;100                           | 0;0                              |
| Clear cell (tubulo) papillary renal cell carcinoma                           | 21               | 17;100                            | 0;0                              |
| Chromophobe renal cell carcinoma                                             | 131              | 71;100                            | 0;0                              |
| Oncocytoma                                                                   | 177              | 97;100                            | 0;0                              |
| Tumor entity                                                                 | CGA / SYN immunostaining | TMA (n) | CGA negative / SYN positive (n,%) | CGA negative / SYN positive (n,%) | CGA positive / SYN positive (n,%) | CGA positive / SYN positive (n,%) |
|-----------------------------------------------------------------------------|---------------------------|---------|-----------------------------------|-----------------------------------|-----------------------------------|-----------------------------------|
| **Tumors of the male genital organs**                                        |                           |         |                                   |                                   |                                   |                                   |
| Adenocarcinoma of the prostate, Gleason 3+3                                | 83                        | 82      | 81.988                             | 0.0                               | 0.0                               | 1.1.2                             |
| Adenocarcinoma of the prostate, Gleason 4+4                                | 80                        | 74      | 64.865                             | 3.4.1                             | 3.4.1                             | 4.5.4                             |
| Adenocarcinoma of the prostate, Gleason 5+5                                | 85                        | 84      | 70.833                             | 3.3.6                             | 9.10.7                             | 2.2.4                             |
| Adenocarcinoma of the prostate (recurrence)                                | 258                       | 168     | 136.81                             | 10.6                              | 13.7.7                             | 9.5.4                             |
| Small cell neuroendocrine carcinoma of the prostate                        | 19                        | 17      | 2.11.8                             | 5.29.4                             | 1.5.9                             | 9.52.9                             |
| Seminoma                                                                   | 621                       | 526     | 526.100                            | 0.0                               | 0.0                               | 0.0                               |
| **Tumors of endocrine organs and neuroendocrine neoplasms**                 |                           |         |                                   |                                   |                                   |                                   |
| Adenoma of the thyroid gland                                               | 114                       | 94      | 94.100                             | 0.0                               | 0.0                               | 0.0                               |
| Papillary thyroid carcinoma                                                 | 392                       | 202     | 200.99                             | 1.0.5                             | 1.0.5                             | 0.0                               |
| Follicular thyroid carcinoma                                                | 154                       | 103     | 103.100                            | 0.0                               | 0.0                               | 0.0                               |
| Medullary thyroid carcinoma                                                 | 111                       | 96      | 0.0                                | 0.0                               | 0.0                               | 4.4.2                             |
| Anaplastic thyroid carcinoma                                                | 45                        | 37      | 37.100                             | 0.0                               | 0.0                               | 0.0                               |
| Adrenal cortical adenoma                                                    | 50                        | 50      | 22.44                              | 24.48                              | 3.6.1                             | 1.2                               |
| Adrenal cortical carcinoma                                                 | 26                        | 24      | 2.8.3                              | 22.91.7                            | 0.0                               | 0.0                               |
| Paraganglioma                                                               | 41                        | 39      | 0.0                                | 4.10.3                             | 0.0                               | 35.89.7                           |
| Phaeochromocytoma                                                           | 50                        | 48      | 0.0                                | 1.2.1                              | 0.0                               | 47.97.9                           |
| Appendix, neuroendocrine tumor (NET)                                       | 21                        | 10      | 0.0                                | 0.0                               | 0.0                               | 10.100                            |
| Colorectal, neuroendocrine tumor (NET)                                     | 12                        | 9       | 0.0                                | 1.11.1                            | 0.0                               | 6.88.9                            |
| Liver, neuroendocrine tumor (NET)                                          | 49                        | 38      | 0.0                                | 0.0                               | 0.0                               | 38.100                            |
| Lung, neuroendocrine tumor (NET)                                           | 19                        | 15      | 0.0                                | 1.6.7                              | 0.0                               | 14.93.3                           |
| Pancreas, neuroendocrine tumor (NET)                                        | 95                        | 85      | 1.1.2                              | 3.3.5                              | 4.4.7                             | 77.90.6                           |
| Colorectal, neuroendocrine carcinoma (NEC)                                 | 12                        | 5       | 0.0                                | 2.4.0                              | 0.0                               | 3.60                              |
| Gallbladder, neuroendocrine carcinoma (NEC)                                | 4                         | 1       | 0.0                                | 0.0                               | 0.0                               | 1.100                             |
| Pancreas, neuroendocrine carcinoma (NEC)                                   | 13                        | 13      | 0.0                                | 2.15.4                             | 0.0                               | 11.84.6                           |
| **Tumors of soft tissue and bone**                                          |                           |         |                                   |                                   |                                   |                                   |
| Tenosynovial giant cell tumor                                               | 45                        | 44      | 44.100                             | 0.0                               | 0.0                               | 0.0                               |
| Granular cell tumor                                                        | 53                        | 11      | 11.100                             | 0.0                               | 0.0                               | 0.0                               |
| Leiomyoma                                                                   | 50                        | 46      | 46.100                             | 0.0                               | 0.0                               | 0.0                               |
| Leiomyosarcoma                                                              | 87                        | 82      | 78.95.1                            | 4.4.9                              | 0.0                               | 0.0                               |
| Liposarcoma                                                                 | 132                       | 61      | 61.100                             | 0.0                               | 0.0                               | 0.0                               |
| Malignant peripheral nerve sheath tumor (MPNST)                            | 13                        | 10      | 10.100                             | 0.0                               | 0.0                               | 0.0                               |
| Myofibrosarcoma                                                             | 26                        | 23      | 23.100                             | 0.0                               | 0.0                               | 0.0                               |
| Angiosarcoma                                                                | 73                        | 20      | 20.100                             | 0.0                               | 0.0                               | 0.0                               |
| Angiomyolipoma                                                              | 91                        | 57      | 57.100                             | 0.0                               | 0.0                               | 0.0                               |
| Dermatofibrosarcoma                                                         | 21                        | 12      | 12.100                             | 0.0                               | 0.0                               | 0.0                               |
| Ganglieneuroma                                                              | 14                        | 9       | 7.77.8                             | 1.11.1                             | 0.0                               | 1.11.1                             |
| Neurofibroma                                                                | 117                       | 96      | 96.100                             | 0.0                               | 0.0                               | 0.0                               |
| Sarcoma, not otherwise specified (NOS)                                      | 74                        | 58      | 58.100                             | 0.0                               | 0.0                               | 0.0                               |
| Ewing sarcoma                                                               | 23                        | 7       | 6.85.7                             | 1.14.3                             | 0.0                               | 0.0                               |
| Rhabdomyosarcoma                                                            | 6                         | 5       | 5.100                              | 0.0                               | 0.0                               | 0.0                               |
| Schwannoma                                                                  | 121                       | 109     | 107.98.2                           | 2.1.8                              | 0.0                               | 0.0                               |
| Synovial sarcoma                                                            | 12                        | 8       | 7.87.5                             | 1.12.5                             | 0.0                               | 0.0                               |
| Osteosarcoma                                                                | 43                        | 11      | 11.100                             | 0.0                               | 0.0                               | 0.0                               |
| Chondrosarcoma                                                              | 38                        | 4       | 4.100                              | 0.0                               | 0.0                               | 0.0                               |
were most commonly positive in neuroendocrine neoplasms including neuroendocrine tumors of the appendix, colorectum, ileum, pancreas and lung, neuroendocrine carcinoma of the colorectum, the gall bladder and the pancreas, medullary thyroid carcinoma, Merkel cell carcinoma, paraganglioma, phaeochromocytoma, and small cell neuroendocrine carcinoma of the bladder, the prostate and the lung. Among these tumors, 96.7% (of n = 460) showed a positive staining for synaptophysin and/or chromogranin.

Among the remaining non-neuroendocrine tumor entities, only 6.3% of n = 9,237 of tumors with available data on both markers were synaptophysin and/or chromogranin A positive including 3.4% of cases with synaptophysin staining (120 weak, 97 moderate, 97 strong), 4.1% with chromogranin A staining (141 weak, 189 moderate, 46 strong), and 1.1% (106 tumors) with staining for both markers. A total of 41 of 88 analyzed non-neuroendocrine tumor entities contained at least one case with either chromogranin A or synaptophysin immunostaining and 22 tumor entities included at least one case with strong positivity of at least one of these markers (Table 1).

Representative images of synaptophysin and chromogranin A positive tumors are shown in Figs. 2–4. It is of note, that many tumors classified as weakly positive only contained few scattered cancer cells with strong expression of synaptophysin and/or chromogranin. This was particularly common in adrenocarcinoma of the prostate, mucinous carcinoma of the ovary, invasive breast carcinoma of no special type, ductal adenocarcinoma of the pancreas, gastric adenocarcinoma and adenocarcinoma of the colon (Fig. 3). A graphical representation of the prevalence of synaptophysin and/or chromogranin A positivity among tumors is given in Fig. 5. In this ranking order, various types of neuroendocrine neoplasms are followed by adrenocortical tumors and basal cell carcinoma of the skin. These latter tumor entities stood out because basal cell carcinomas expressed only chromogranin A and adrenocortical tumors only synaptophysin. A subdivision of non-neuroendocrine neoplasms revealed “neuroendocrine differentiation” most commonly in adenocarcinomas from the female genital tract (18.9% of 750), pancreatic-/hepato-/biliary origin (15.8% of 497), the prostate (14.9% of 408), the upper gastrointestinal tract (8.9% of 349), lower gastrointestinal tract (6.8% of 1249), breast (6.3% of 1393), and the lung (3.0% of 166) while it was particularly rare in urothelial carcinomas (1.0% of 724) and squamous cell carcinomas (0.6% of 469).

Synaptophysin and chromogranin A expression, tumor phenotype and prognosis. Synaptophysin and chromogranin A immunostaining was not associated with parameters of disease aggressiveness in 204 evaluable endometrium cancers, 249 evaluable pancreatic adenocarcinomas, 233 evaluable gastric adenocarcinomas and 1,182 evaluable colorectal adenocarcinomas. In a cohort of 1,073 breast carcinomas of no special type, synaptophysin positivity was seen in 4.9% of cases and was linked to advanced tumor stage (p = 0.0427), high tumor grade (p = 0.0319) and loss of estrogen receptor expression (p = 0.0061). In the same cohort of breast cancers chromogranin A was only linked to loss of estrogen receptor expression (p = 0.0213). There was no association with overall survival.

**Fig. 2.** Synaptophysin and chromogranin A immunostaining in tumors. (A) Strong diffuse synaptophysin staining in an adrenocortical carcinoma. (B) Moderate and patchy synaptophysin staining in an adrenocortical carcinoma of the lung. (C) Moderate synaptophysin staining in an endometrioid carcinoma of the ovary (D) Moderate diffuse synaptophysin staining in a serous carcinoma of the ovary. (E) Moderate diffuse chromogranin A staining in an invasive breast carcinoma of no special type. (F) Strong and mostly diffuse chromogranin A staining in an endometrioid endometrial carcinoma. (G) Moderate to strong and patchy chromogranin A staining in an endometrioid carcinoma of the ovary. (H) Moderate diffuse chromogranin A staining in a basal cell carcinoma.
4. Discussion

The successful immunhistochemical analysis of 9,697 tumors identified neuroendocrine marker expression in 10.6% (n = 1,029) of tumors. While about half of tumors with synaptophysin or/and chromogranin A positivity belonged to neuroendocrine tumor entities, neuroendocrine markers were also seen in 6.3% (n = 584) of 9,237 tumors from non-neuroendocrine entities. Basal cell carcinomas and adrenocortical tumors were the most frequently positive non-neuroendocrine tumors in our study. These tumors are unique because unlike all other neoplasms with neuroendocrine features, basal cell carcinomas expressed only chromogranin A (in 50.0% of our 64 analyzable cases) and adrenocortical carcinomas expressed only synaptophysin (in 91.7% of our 24 analyzable cases). These observations fit with previous data and may argue for a specific function of the respective proteins in these tumors rather than reflecting "neuroendocrine differentiation". Previous studies showed chromogranin A positivity in basal cell carcinoma between 27 and 72% (Houcine et al., 2017; Terada, 2013) and synaptophysin positivity in 67%–85% of adrenal cortical neoplasms (Weissferdt et al., 2014; Sangoi and McKenney, 2010; Komminoeth et al., 1995; Miettinen, 1992). Synaptophysin or chromogranin A expression can lead to diagnostic difficulties in these tumors. For example, chromogranin A positive basal cell carcinoma may be confused with Merkel cell carcinoma of the skin or metastatic small cell carcinoma, especially on small biopsies (Pollock et al., 2019). Because of its high positivity rate in adrenocortical cells, synaptophysin staining should not be used in the differential diagnosis with phaeochromocytoma (2017).

Features of neuroendocrine differentiation were identified in tumors from 41 of 88 different non-neuroendocrine entities analyzed in this study. These findings are consistent with the existing literature as low fractions of cases with "neuroendocrine differentiation" have been described in many different tumor entities. Tumors particularly well known for occasional expression of neuroendocrine markers include for example prostate cancer (reviewed in (Puca et al., 2019)), breast cancer (reviewed in (Tsang and Tse, 2021)), colon cancer (reviewed in (Kleist and Poetsch, 2015)), ovarian carcinoma (Taube et al., 2015; Sasaki et al., 1989) and endometrial carcinomas (Moritz et al., 2019), as well as non-epithelial tumors, such as Ewing sarcoma (Machado et al., 2021). That chromogranin A and synaptophysin positivity was largely unrelated to histopathological features of cancer aggressiveness in endometrium cancers, pancreatic adenocarcinomas, gastric adenocarcinomas and colorectal adenocarcinomas in this study further argues against a clinical importance of neuroendocrine differentiation as long as a tumor retains its typical morphology.

In prostate cancer, neuroendocrine differentiation is being viewed as
a marker of disease progression, especially in response to androgen deprivation (Hirano et al., 2004; Berruti et al., 2007; Mucci et al., 2000). Consistent with this notion, a higher percentage of neuroendocrine differentiation was found among recurring adenocarcinoma of the prostate (19.0% of n = 168) and cancers with Gleason 5+5 (16.7% of n = 84) compared to hormone-naïve cancers Gleason 3+3 and Gleason 4+4 tumors (7.1% of n = 156). However, that only 19.0% of cancers recurring under anti-androgen therapy expressed neuroendocrine markers demonstrates that this feature is not a very sensitive marker for prostate cancer progression. Given that 7.1% of our untreated 3+3 and 4+4 cancers also expressed neuroendocrine markers shows that this feature also lacks specificity for progressive disease. Since most studies evaluating clinical endpoints failed to find any clinical significance of neuroendocrine marker expression in hormone-naïve tumors, the immunohistochemical analysis of neuroendocrine markers has not been recommended for general use of acinar adenocarcinoma of the prostate in the current WHO Classification of Tumors of the Urinary System and Male Genital Organs (2022).

That single cell neuroendocrine marker positivity was particularly frequent in colorectal cancer fits with data from previous studies...
describing neuroendocrine marker-positive cells in up to 40% of colorectal adenocarcinomas (Ogini et al., 2019; Foley et al., 1998). The same phenomenon has been described in several other cancers including esophageal (Hamilton et al., 2000) and gastric adenocarcinoma (Wang et al., 2013). It is of note that 11% of our 100 gastric adenocarcinomas of the diffuse type showed a moderate to strong staining for synaptophysin and/or chromogranin A. This is consistent with a development of at least a fraction of these tumors from enterochromaffine-like (ECL) cells, another neuroendocrine cell-type (Prinz et al., 2003). An ECL origin of these tumors has been suggested by several authors based on RNA and protein expression data (Waldhum and Mjones, 2020; Bartley et al., 2011; Fujiyoshi and Eimoto, 2008; Bakkeland et al., 2006). From a diagnostic point of view, it is important, that single neuroendocrine marker-positive cells within the tumor glands are not confused with a mixed neuroendocrine-non-neuroendocrine neoplasm (MiNEN), which is mostly made of a poorly differentiated NEC component, together with an adenocarcinoma component.

Given the size of our study, it is not surprising that positive cases were also found in the tumor entities for which neuroendocrine differentiation has so far not been described. For example, uterine leiomyosarcoma showed unexpected neuroendocrine differentiation in 4.9% of 82 analyzed cases. Although studies on synaptophysin or chromogranin expression in leiomyosarcomas do not exist, our data are consistent with frequent expression of the much less specific “neuroendocrine marker” CD56 in most uterine smooth muscle tumors (Karpathiou et al., 2021). The fact that in many tumor entities neuroendocrine marker positivity was only found in a single case may suggest, that synaptophysin or chromogranin A positivity can - in principle – occur in every tumor entity. Whether or not such findings reflect a biologically relevant neuroendocrine differentiation of tumor cells is uncertain.

It is the pivotal strength of our study that TMAs were used since this approach enables the analysis of an unprecedented number of tumors and offers maximum standardization of analysis. Standardization not only affects the staining procedures, but also the quantity of tissue analyzed per patient and the time between cutting and staining a section (section storage time). Studies have shown that section storage time of only two weeks results in substantial reduction of immunostaining (Mirlacher et al., 2004; Jacobs et al., 1996). It would be very difficult to carry out a study with over 10,000 tumors with identical staining procedures for each case. It is of note, that the age of the tissue blocks is another neuroendocrine cell-type (Prinz et al., 2003). An ECL origin of neuroendocrine differentiation has so far not been described. For example, uterine leiomyosarcoma showed unexpected neuroendocrine differentiation in 4.9% of 82 analyzed cases. Although studies on synaptophysin or chromogranin expression in leiomyosarcomas do not exist, our data are consistent with frequent expression of the much less specific “neuroendocrine marker” CD56 in most uterine smooth muscle tumors (Karpat-hiou et al., 2021). The fact that in many tumor entities neuroendocrine marker positivity was only found in a single case may suggest, that synaptophysin or chromogranin A positivity can - in principle – occur in every tumor entity. Whether or not such findings reflect a biologically relevant neuroendocrine differentiation of tumor cells is uncertain.

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Moreover, the amount of tissue analyzed on a TMA is comparable to the amount of tissue analyzed on a small biopsy, the typical material for a primary diagnosis of a neuroendocrine neoplasm. The major limitation of our TMA study is, that although we analyzed over 14,000 tumors, some tumor entities are underrepresented.

In summary, our extensive analysis of over 103 different tumor types and subtypes show that “neuroendocrine differentiation” is more common than expected. Evidence for a strong link between “neuroendocrine differentiation” and tumor aggressiveness could not be found.

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CRediT authorship contribution statement

David Dum: Conceptualization, Data curation, Formal analysis, Writing - original draft. Natalia Gorbokon: Conceptualization, Data curation, Formal analysis. Anne Menz: Data curation, Formal analysis. Franziska Bäschke: Data curation, Resources, Validation. Andreas M. Luebbe: Data curation, Resources, Validation. Andrea Hinsch: Data curation, Resources, Validation. Katharina Möller: Data curation, Validation. Christian Bernreuther: Data curation, Visualization. Patrick Lebok: Conceptualization. Sören Weidemann: Conceptualization. Frank Jacobsen: Conceptualization. Till S. Clauditz: Conceptualization. Stefan Steurer: Conceptualization. Rainer Krech: Conceptualization.

Declaration of competing interest

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Appendix A. Supplementary data

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