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Expression of neuroendocrine markers: a signature of human undifferentiated carcinoma of the colon and rectum

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Abstract The frequency and prognostic significance of neuroendocrine marker expression in undifferentiated colorectal cancers has not yet been studied in great detail. Therefore, the survival of 20 patients with small cell undifferentiated colorectal cancers, treated at our institution between 1982 and 1997 (0.8% of all operated colorectal carcinomas), was correlated with the extent of neuroendocrine differentiation. Chromogranin A, synaptophysin, syntaxin1, VAMP2, SNAP25 and α/β-SNAP were used as neuroendocrine markers. Based on the degree of immunoreactivity for these marker proteins, tumors were separated into group 0 (<2% cells stained positive for neuroendocrine markers) and group 1 (>2% cells stained positive). Patients were followed up for at least 5 years or until death. Of 20 (45%) undifferentiated colorectal tumors, 9 expressed neuroendocrine markers (group 1). Only one patient of this group survived for 2 years (11%), whereas the 2-year-survival rate was 45.4% in group 0. Of the 11 patients in group 0, 9 were diagnosed with UICC stages I–III, whereas 8 of 9 tumors with expression of neuroendocrine markers were diagnosed with UICC stage IV (P=0.002). Our results show that neuroendocrine differentiation is often seen in small cell undifferentiated colorectal cancer. It correlates with a more aggressive course of the disease.

Keywords Neuroendocrine differentiation · Prognostic marker · SNARE · Synaptophysin · Undifferentiated colorectal carcinoma

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Introduction

Neuroendocrine cells are found among epithelial cells in most surfaces of the body. They share many morphological, biochemical and functional similarities with neuronal cells such as a polarized membrane orientation, neurotransmitter-synthesizing enzymes, neural cell adhesion molecules, and peptide and amino acid transmitter receptors (for review see [44]). Similar to neurons, neuroendocrine cells possess a complete molecular machinery for the uptake and release of neurotransmitters and the secretion of neuropeptides [45]. These substances are stored within membrane-bound granules or vesicles, from which they are released in response to a stimulus. Two vesicle-types are known. First, large dense-core vesicles (100–400 nm in diameter) are characterized immunohistochemically by chromogranin A, a matrix protein of neuroendocrine granules [35]. Second, small synaptic vesicle analogs (40–80 nm in diameter) are characterized immunohistochemically by synaptophysin, an integral protein of the vesicle membrane [37]. The process of synaptic vesicle docking and/or fusion relies on a highly conserved protein complex (SNARE complex), consisting of the NSF protein (*N*-ethylmaleimide-sensitive factor) and α/β-SNAPs (soluble NSF-associated proteins) as well as the two synaptic vesicle membrane proteins VAMP2 (vesicle-associated membrane protein) and synaptotagmin, and the two synaptic plasma membrane proteins syntaxin1 and SNAP25 (synaptosomal-associated protein) [14, 29]. Since neuroendocrine cells pertain to small synaptic vesicle analogs regardless of their state of differentiation – in contrast to large dense

core granules, which are often lost during cellular dedifferentiation – proteins of small synaptic vesicles represent universal permanent markers for neuroendocrine cells [43].

The histogenesis of neuroendocrine differentiated carcinomas in general and of gastrointestinal neuroendocrine cells in particular has been a matter of debate. Neuroendocrine cells in this location were initially thought to be of neural crest origin [25]. Le Douarin, however, provided evidence that neuroendocrine cells of the gut are derived from the endoderm [19]. Cell lineage studies support the notion that gut endocrine cells share the same clonal origin as the other cell types of the gut do. Thus, a single epithelial cell, albeit malignant, can give rise to all cell types seen in the colorectal epithelium [17, 46].

Tumor cells with neuroendocrine differentiation have been known for many decades to be present in gastrointestinal carcinomas [18, 36]. We previously showed that neuroendocrine differentiation of colorectal adenocarcinomas is an independent unfavorable prognostic factor in stage-III and -IV disease [7].

Small cell undifferentiated carcinomas (SCUCs) of the colon and rectum are rare and morphologically resemble those of the lung [16] and elsewhere [13, 28]. Among the wide histopathologic spectrum of colorectal cancers, SCUCs are the most aggressive neoplasms, characterized by early dissemination and a rapid clinical deterioration [32]. Tumor cells with neuroendocrine differentiation are often seen in SCUCs. The first documented series of colonic SCUCs with neuroendocrine features was reported by Gould and Chejfec in 1978 [6] and sporadic reports have appeared since, primarily in the form of small series or case reports. The neuroendocrine differentiation has been based on ultrastructural and/or immunohistochemical studies. To our knowledge 59 well-documented colorectal small cell carcinomas with neuroendocrine features have been published in the English language literature so far [3, 5, 6, 12, 20, 23, 26, 27, 32, 34, 39, 41, 42]. The presence of neuroendocrine differentiation hereby appears to be associated with a poorer prognosis [5], as described before in their adenocarcinoma counterparts [7]. Nevertheless, the frequency of neuroendocrine differentiation of SCUCs remains elusive, since all previous reports focussed on undifferentiated carcinomas with neuroendocrine features. In addition, the prognostic significance of neuroendocrine cells within undifferentiated colorectal tumors has not been defined yet.

Here, we report on the frequency and prognostic value of the expression of neuroendocrine markers in a series of undifferentiated colorectal cancers. In order to better evaluate the status of proteins of the regulated secretory pathway in this subgroup of tumors, we applied not only markers of large dense core vesicles such as chromogranin A or of small synaptic vesicle analogs such as synaptophysin but also markers of the SNARE complex (syntaxin1, VAMP2, SNAP25 and α/β-SNAP).

Materials and methods

Patients

Twenty cases of SCUCs were observed at the Benjamin Franklin University Clinics, Berlin, between 1982 and 1997, and were recovered from the routine pathological files. Eleven poorly differentiated adenocarcinomas (PDACs) of the colon and rectum of the same time period served as controls. All tumors were surgically resected. Clinical information and follow-up data were obtained from the hospital records, the patients' primary doctors, and the official federal registration office. Patients were followed up for at least 5 years or until death. Tumor staging was complete in all cases and was applied according to the UICC (Union International Contre le Cancer) staging system. Within the 'undifferentiated carcinoma' group, there were two patients diagnosed with stage-II (1 woman, 1 man), eight patients (2 women, 6 men) with stage-III, and 10 patients (3 women, 7 men) with stage-IV colorectal cancer. The mean age was 58.3 years (range 27–83 years). The primary site of the tumors was classified as rectum (n=7) or colon (n=13), with a tendency to right-sided tumors (9 of 13 colonic tumors). Depth of tumor invasion was assessed and recorded as T2 (n=2), T3 (n=12) or T4 (n=6). Nine patients had liver metastases at the time of diagnosis, one presented with brain metastasis, and one with peritoneal carcinosis. One patient died immediately after surgery and autopsy was performed. Besides the liver metastases, metastases in the lungs, pancreas, bone marrow, and in multiple lymph nodes (including subclavicular lymph nodes) were present. This patient was excluded from statistical survival analysis. Some of the patients received adjuvant (n=3) or palliative (n=6) chemotherapy, six patients received pre- or postoperative radiotherapy.

The 11 patients with PDAC of the colon and rectum (9 women, 2 men) were diagnosed with stage-II (n=4), stage-III (n=3), and stage-IV disease (n=4), respectively. The mean age was 69.1 years (range 51–83 years). In this group, we found five rectal and six colonic carcinomas (3 of 6 right-sided). Depth of tumor invasion was recorded as T3 (n=9) or T4 (n=2). Four patients presented with liver metastasis at the time of diagnosis. In this group, two patients received adjuvant, one patient palliative chemotherapy; one patient underwent postoperative radiotherapy.

Tumor type

Thirty-one cancer specimens were examined. Histologically, the neoplasms were classified as pure undifferentiated carcinomas (n=19, specimen no. 1–19), undifferentiated carcinoma in association with adenocarcinoma (n=1, specimen no. 20) or as PDAC (n=11, specimen no. 21–31). When present, the adenocarcinoma component consisted of invasive tumor with characteristic glandular architecture (specimen no. 20–31). In the biphenotypic case, the undifferentiated carcinoma component and the adenocarcinoma component occurred as separate masses with distinct morphology. Mitoses were numerous in all tumors.

Clinicopathological features of all patients are summarized in Table 1.

Immunohistochemistry

Representative samples of the tumors were routinely processed and embedded in paraffin. Sections (2–3 μm) of the tumor, through its most invasive part and including adjacent normal mucosa, were stained using standard immunohistochemical techniques. Antibodies against the antigens studied, their manufacturers, and working dilutions are listed in Table 2. Detection of bound primary monoclonal antibodies was assessed using the alkaline phosphatase anti-alkaline phosphatase (APAAP) complex method as described elsewhere [7]. Neuroendocrine cells of the

Table 1 Characteristics of 20 small cell undifferentiated and 11 poorly differentiated carcinomas of the colorectum. Specimens no. 1–19: small cell, undifferentiated colorectal carcinoma. Specimen no. 20: biphenotypic tumor with adenocarcinoma and undifferentiated carcinoma. Specimen no. 21–31: *PDAC* poorly differentiated adenocarcinoma

Specimen no.	Age (years)/Sex	Tumor location	Histologic subtype	TNM classification	Metastasis at presentation
1	51, Female	Sigmoid	Undifferentiated	pT4 N0 M0	
2	79, Female	Cecum	Undifferentiated	pT4 N3 M0	
3	83, Female	Ascending colon	Undifferentiated	pT3 N1 M0	
4	50, Male	Ascending colon	Undifferentiated	pT4 N0 M0	
5	72, Male	Rectum	Undifferentiated	pT3 N2 M0	
6	83, Female	Ascending colon	Undifferentiated	pT3 N1 M1	Liver
7	70, Male	Transverse colon	Undifferentiated	pT3 N2 M0	
8	59, Male	Right flexure	Undifferentiated	pT4 N1 M1	Liver
9	68, Male	Rectum	Undifferentiated	pT3 N1 M0	
10	58, Male	Descending colon	Undifferentiated	pT4 N1 M0	
11	27, Male	Rectum	Undifferentiated	pT3 N2 M0	
12	81, Female	Rectum	Undifferentiated	pT3 N2 M1	Liver
13	69, Male	Transverse colon	Undifferentiated	pT3 N1 M1	Liver
14	49, Male	Sigmoid	Undifferentiated	pT2 N2 M1	Liver
15	32, Male	Ascending colon	Undifferentiated	pT3 N2 M0	
16	29, Male	Rectum	Undifferentiated	pT2 N2 M1	Liver
17	63, Female	Right flexure	Undifferentiated	pT4 N3 M1	Liver, lung, pancreas
18	57, Male	Rectum	Undifferentiated	pT3 N1 M1	Liver
19	32, Male	Sigmoid	Undifferentiated	pT3 N0 M1	Liver, brain
20	55, Male	Rectum	Undifferentiated PDAC	pT3 N3 M1	Liver
21	79, Female	Descending colon	PDAC	pT4 N1 M1	Liver
22	72, Female	Ascending colon	PDAC	pT3 N0 M0	
23	75, Female	Rectum	PDAC	pT3 N0 M0	
24	55, Female	Cecum	PDAC	pT3 N0 M0	
25	51, Female	Rectum	PDAC	pT3 N2 M0	
26	66, Male	Rectum	PDAC	pT3 N0 M1	Liver
27	68, Female	Rectum	PDAC	pT4 N1 M1	Liver
28	83, Female	Sigmoid	PDAC	pT3 N1 M0	
29	83, Male	Left flexure	PDAC	pT3 N0 M0	
30	69, Female	Ascending colon	PDAC	pT3 N1 M1	Liver
31	59, Female	Rectum	PDAC	pT3 N1 M0	

Table 2 Immunohistochemical reagents and dilutions used

Reagent		Source	Dilution
Anti-synaptophysin (SY38)	Monoclonal	Biogenex, San Ramon, Calif., USA	1:25
Anti-chromogranin (LK2H10)	Monoclonal	Linaris, Wertheim-Biettingen, Germany	1:4
Anti-α/β-SNAP	Monoclonal	Synaptic Systems, Göttingen, Germany	1:500
Anti-syntaxin1	Monoclonal	Synaptic Systems, Göttingen, Germany	1:1250
Anti-SNAP 25	Monoclonal	Synaptic Systems, Göttingen, Germany	1:750
Anti-VAMP2	Monoclonal	Synaptic Systems, Göttingen, Germany	1:1000
Anti-ki67 (MIB-1)	Monoclonal	Dako, Germany	1:1000

non-neoplastic colonic mucosa and of pancreatic islet cells served as positive control of the immunoreaction. Negative controls were obtained by substituting the immunoglobulin fraction of a mouse serum for the primary antibody.

The stained slides were first independently evaluated by the authors P. Grabowski and J. Schönfelder; if the evaluation provided differing results, a consensus interpretation was reached with the pathologist H.D. Foss. Overall, the degree of inter-observer variation between these investigators was less than 10%. The number of immunoreactive tumor cells for neuroendocrine marker proteins was evaluated by examining three representative high power fields (400×). Based on the degree of immunoreactivity, tumors were separated into group 0 (<2% cells stained positive for neuroendocrine markers) and group 1 (>2% cells stained positive). As normal colonic mucosa contains up to 2% neuroendocrine cells, a 2% cut-off was chosen [7]. The nuclear ki67/MIB-1 labeling index was expressed as the percentage of positively stained cells with respect to a hundred cells in ten high-power fields.

Statistics

Correlations between the data of the various groups were investigated using the Chi-square test (dichtiomised parameter) or Mann-Whitney U-test (for non-dichtiomised parameters). Univariate analysis to detect influencing parameters on the survival of the various groups was performed using the Cox's proportional hazards regression model with SPSS software (Statistical package model with SPSS software (Statistical package of social science; Institute of Chicago, Ill.). Differences were considered to be significant for $P<0.05$.

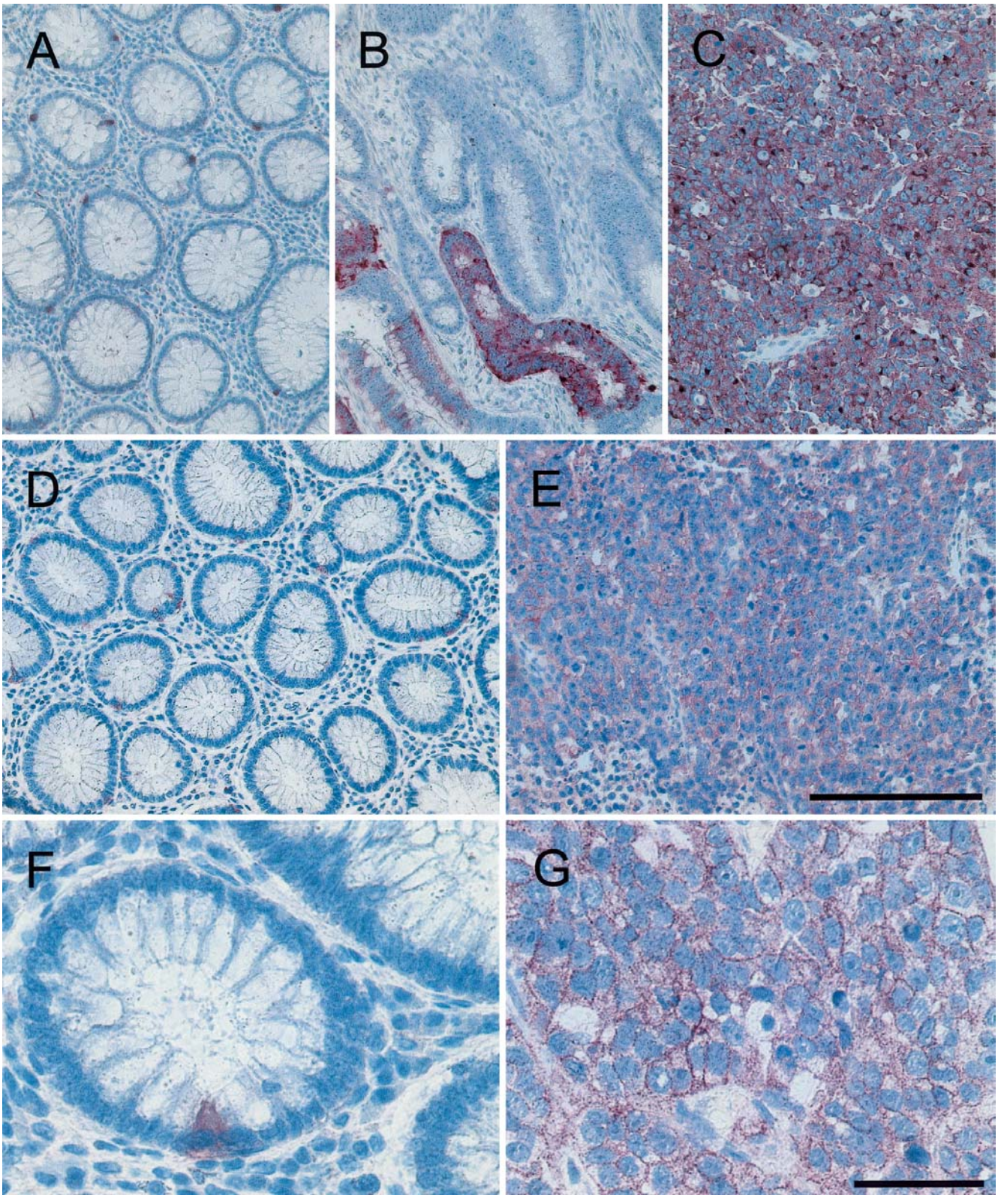

Fig. 1 Representative areas from the biphenotypic tumor (specimen no. 20). Immunohistochemistry for detection of synaptophysin positive cells in (**a**) normal mucosa (<2% of all cells stain positive), (**b**) poorly differentiated adenocarcinoma component (about 25% of all tumor cells stain positive), and (**c**) undifferentiated carcinoma component; note strong uniform staining in this part of the tumor. Immunohistochemistry for detection of α/β-SNAP in (**d**) normal mucosa and (**e**) undifferentiated carcinoma component; note the same staining pattern as in (**c**). *Scale bar* 200 μm. Immunohistochemistry for detection of SNAP25 in (**f**) normal mucosa (localization: membranous) and (**g**) undifferentiated carcinoma component (localization: membranous and cytoplasmic). *Scale bar* 50 μm

Table 3 Antigenic profiles of undifferentiated colorectal carcinoma with neuroendocrine features (specimens no. 12–19), one biphenotypic tumor with adenocarcinoma and undifferentiated carcinoma (specimen no. 20), and poorly differentiated colorectal carcinomas (PDAC, specimens no. 22 and no. 31). – no staining, + less than 10% of cells stained, ++ 10–50% of cells stained, +++ greater than 50% of cells stained. Specimen numbers are the same as in Table 1

Specimen no.	Histologic subtype	CgA	Synaptophysin	a/b-SNAP	SNAP25	VAMP2	Syntaxin1
12	Undifferentiated	+	+++	+++	+++	–	–
13	Undifferentiated	–	+++	+++	–	–	+++
14	Undifferentiated	–	+++	+++	+++	++	+++
15	Undifferentiated	+++	+++	+++	+++	–	+++
16	Undifferentiated	++	+++	+++	+++	+	+++
17	Undifferentiated	–	++	++	–	–	++
18	Undifferentiated	–	+++	+++	++	–	++
19	Undifferentiated	++	+++	+++	+++	+++	+++
20	Undifferentiated	+++	+++	+++	+++	+	+++
	PDAC	+	++	+	+	+	+
22	PDAC	–	++	++	+	+	–
31	PDAC	+	++	+	–	–	+

Results

Immunohistochemistry of neuroendocrine markers in SCUCs

All six examined neuroendocrine markers (chromogranin A, synaptophysin, α/β-SNAP, SNAP25, VAMP2, and syntaxin1) were detected in non-neoplastic colonic mucosa of all 20 resection specimens (Fig. 1a, d, f). The extent of expression was lower than 2% of all colonic mucosal cells. Within neoplastic tissue, neuroendocrine cells were detected in 9 (45%) of the 20 undifferentiated colorectal carcinomas (group 1). Apart from one tumor with biphenotypic features, studying different areas of the same tumor did not yield significant differences, since almost all tumor cells showed the same expression pattern.

Synaptophysin versus other neuroendocrine marker expression

In non-neoplastic colonic mucosa, the distribution of synaptophysin immunoreactivity cells was similar to the pattern of chromogranin A, α/β-SNAP, SNAP25, VAMP2, and syntaxin1 staining. Similarly, all tumors of group 1 that showed positive immunoreactivity for the above-mentioned markers also displayed synaptophysin staining. However, chromogranin A was only expressed in 5 of 9 tumors, VAMP2 in 4 of 9, SNAP25 in 7 of 9 and syntaxin1 in 8 of 9 tumors, whereas all nine tumors expressed α/β-SNAP (Table 3). The reason for the differential expression of these marker molecules of the regulated secretory pathway may be due to the loss of some of these proteins during dedifferentiation.

Comparison of SCUCs with PDACs

All 11 PDACs displayed a glandular growth pattern as seen typically in adenocarcinomas. Mitoses were numerous in all tumors, albeit less frequent than in the SCUC subgroup (average proliferation index 50% versus 70%, respectively). Two tumors were found to display an expression of neuroendocrine marker proteins (group 1). The marker molecules of the regulated secretory pathway were again differentially expressed (Table 3). Interestingly, the expression of neuroendocrine markers found in adenocarcinomas never reached more than 50% of all tumor cells, as we have seen before in our previous study [7]. When neuroendocrine features were seen in undifferentiated colorectal carcinomas, the expression was almost 100% in each case, suggesting that the malignant neuroendocrine cells are so aggressive that they overgrow any initial adenomatous component of the tumor. A decent example of this hypothesis is the biphenotypic tumor (specimen no. 20), with a partly neuroendocrine differentiation (about 25% expression of all marker molecules tested) in the adenomatous component (Fig. 1b), and a 100% expression of synaptophysin, chromogranin A and all SNARE-proteins in the undifferentiated part of the tumor (Fig. 1c, e, g).

Neuroendocrine features and clinicopathological parameters in SCUC

There were no significant differences between group 0 and group 1 in terms of sex, location (colon versus rectum), depth of tumor invasion (T2–3 versus T4), or lymph-node metastasis (N0–1 versus N2; chi-square test). The nine tumors of group 1 were diagnosed more frequently in stage-IV tumor disease (8 of 9 tumors, P=0.002); undifferentiated tumors without neuroendocrine features tended to be diagnosed at an earlier stage (9 of 11 in tumor stages UICC I–III). In addition, neuroendocrine differentiation was observed more frequently among the younger patients (average age at diagnosis 51.9 years in SCUCs with neuroendocrine features versus 63.6 years in SCUCs without neuroendocrine features).

Prognostic implications of neuroendocrine differentiation in SCUC

Two years after tumor surgery, only 1 of the 9 patients with neuroendocrine differentiated SCUC (group 1) had survived (11%), whereas 5 of 11 (45%) group-0 patients were still alive. Certainly, these data may well reflect the different tumor stages at diagnosis: 8 of 9 undifferentiated tumors with neuroendocrine features were diagnosed at UICC stage IV, whereas 9 of 11 undifferentiated tumors without neuroendocrine features were diagnosed at UICC stages I–III. Nevertheless, it is possible that the patients suffering from SCUCs with neuroendocrine features were diagnosed so late, because their colorectal tumors grew so aggressively and fast.

The variables of age, sex, localization of the tumor, stage, depth of tumor infiltration, lymph-node involvement, proliferation index and neuroendocrine markers were analyzed using the univariate Cox's proportional hazards regression model. Due to the small case numbers, no statistically significant results were found.

Discussion

Small cell neuroendocrine carcinoma of the colorectum is classified as high-grade malignant neuroendocrine carcinoma according to WHO [2, 9]. The data from our study confirm the aggressive nature of this tumor type and support the WHO classification. In addition to high-grade malignant (undifferentiated) neuroendocrine carcinomas, the spectrum of neuroendocrine neoplasms includes slowly growing, well-differentiated benign neuroendocrine tumors, and low-grade malignant (well-differentiated) carcinomas of various organs. The origin and development of neuroendocrine tumors or carcinomas has been a matter of debate [1, 5, 6]. Neuroendocrine tumors and low-grade neuroendocrine carcinomas are now believed (by some authors) to arise from orthotopic neuroendocrine cells of the epithelium after damage in partially differentiated precursor cells occurred [1, 20], whereas high-grade neuroendocrine carcinomas are thought to originate from a putative stem cell [10]. Thus, due to their different origin, high-grade neuroendocrine carcinomas, independent of their site of origin, should be clearly distinguished from low-grade neuroendocrine carcinomas and benign neuroendocrine tumors [31].

It is well known that undifferentiated, neuroendocrine carcinomas of the colon and rectum are frequently associated with villous adenoma, adenocarcinoma or adenocarcinomatous elements [20, 32, 39, 42]. Vortmeyer et al. [41] studied the genetic background of synchronous small cell neuroendocrine carcinomas and associated adenocarcinomas of the colorectum and found identical genetic alterations in both tumor components, e.g. a frequent LOH (loss of heterozygosity) for *p53*, *DCC* and *APC* tumor-suppressor genes. In the same study, no such abnormalities were observed in four well-differentiated neuroendocrine tumors. Such findings suggest common genetic changes of the adenoma–carcinoma sequence of PDAC and high-grade neuroendocrine carcinoma that possibly originated from the same stem cell. Reports of scattered neuroendocrine cells in colonic adenocarcinomas and of amphicrine carcinomas with both exocrine and endocrine characteristics in the same cell support this concept [4].

In our study 11 of 20 undifferentiated colorectal cancers stained negative for a broad battery of neuroendocrine markers (group 0). These tumors may represent dedifferentiated adenocarcinomas. However, the 9 carcinomas of group 1 may have originated from a multipotential epithelial stem cell that underwent biphenotypic differentiation after carcinogenesis was initiated. The hypothesis of biphenotypic differentiation implies that a partial neuroendocrine differentiation of the adenocarcinoma was present initially [7], but due to the aggressiveness of the highly malignant neuroendocrine component, the adenocarcinoma was finally outgrown by the neuroendocrine phenotype. Most interesting in this context is one biphenotypic tumor in our study (specimen no. 20) that supports this concept: the adenocarcinoma component exhibited a partial neuroendocrine differentiation (about 25% of the tumor cells, Fig 1b), as evidenced by staining for all six neuroendocrine markers used. However, the undifferentiated component exclusively consisted of neuroendocrine markers expressing cells (Fig. 1c, e, g).

The true prevalence of neuroendocrine SCUC is difficult to determine, since most previous reports described individual case reports or very small series. The only publication presenting a large group of patients (988 colorectal cancers resected at a single institution in Chicago, USA, over 10 years) proposed an incidence of 3.9% for 'neuroendocrine cancers of the colon and rectum' [30]. Since this study included a large number of PDAC as well as 'well-differentiated neuroendocrine carcinoma', the incidence of SCUC with neuroendocrine features may have been 1% or below. According to a publication by the National Cancer Institute, USA [38], only 0.3% of all colorectal malignancies are SCUC. In our hospital, 2530 colorectal carcinomas of any stage were operated on between 1982 and 1997. Reviewing the pathology files of these patients, we found the described 20 SCUC (0.8% of all 2530 cancers). The differences may be due to differing groups of patients being referred to hospital centers. Nevertheless, the frequency of neuroendocrine differentiation of SCUCs remains elusive, since all previous reports [3, 5, 6, 12, 20, 23, 26, 27, 32, 34, 39, 41, 42] focussed on undifferentiated carcinomas with neuroendocrine features, based on ultrastructural or immunohistochemical findings. In our study, however, we found only 9 of 20 (45%) undifferentiated carcinomas to express neuroendocrine marker proteins, based on the immunohistochemical detection of up to six specific neuroendocrine markers. When combining our 9 cases with the previously reported 59 cases of colorectal small cell carcinomas with neuroendocrine features, the average age at diagnosis was 51.3 years (range

26–89 years) and thereby 10 years earlier than colorectal cancer in general, there was no obvious sexual predisposition (29 female, 39 male). Thirty-nine (57%) colorectal small cell carcinomas arose in the rectosigmoid, twenty-five (37% being high for this location) in the coecum/ascending colon, and only four (6%) in the transverse colon. Considering tumor stage, 65 of the 68 current cases (95%) presented with evidence of either regional lymph-node involvement (UICC stage III: 20%) or distant metastases (UICC stage IV: 76%) at the time of diagnosis. In comparison, of the 149,965 colorectal adenocarcinomas reviewed by Thomas and Sobin of the National Cancer Institute [38], 50% had stage-III and 20% had stage-IV disease at the time of diagnosis. In addition, the prognosis of colorectal neuroendocrine carcinoma appears worse than that of stage-matched colorectal adenocarcinomas. Thomas and Sobin [38] noted a 27% 5-year survival when combining stage-III (48.6% 5-year survival) and stage-IV (5.6% 5-year survival) colorectal adenocarcinomas. In contrast, only 3 of 51 patients (of whom the follow-up data were available) with stage-III and stage-IV undifferentiated neuroendocrine carcinomas were alive after 2 years.

The partial neuroendocrine differentiation of otherwise typical adenocarcinomas of the colon and rectum has been shown to be an independent prognostic factor in stage III–IV cancers [7]. The results of our present study in SCUC support the hypothesis that neuroendocrine features correlate with a more aggressive course of the disease. The 11 patients with SCUC and no expression of neuroendocrine markers (group 0) were mostly diagnosed in earlier stages (9 of 11 tumors in UICC stages I–III) and had a 2-year survival rate of 45.4%, whereas only 1 (11%) of the 9 patients of group 1 survived 2 years. Interestingly, 8 of 9 tumors of the latter group were diagnosed in tumor stage IV (P=0.002).

The underlying mechanism for the aggressiveness of these neuroendocrine cancer cells is still not clear. It has been postulated that neuroendocrine tumor cells can stimulate growth through secretion of neurohumoral substances. It has been shown that biogenic amines and polypeptide hormones play a role in growth regulation of normal and neoplastic intestinal epithelium [15]. Expression of these neurotransmitters and neuropeptides and their receptors in cancer cells might constitute an autocrine or paracrine growth-promoting loop that could account for the poor survival.

The secretion of both amines and peptide hormones requires the expression of the SNARE complex, the core proteins of the exocytotic apparatus shared by neuroendocrine cells and neurons. Interestingly, the expression of several SNARE proteins appears to be maintained during neoplastic transformation. Recently, mRNA and protein of SNAP25 and syntaxin1 were detected in small cell lung carcinomas [8]; proteins of synaptophysin, VAMP2, and SNAP25 were found in pheochromocytomas [11] and in different high-grade malignant neuroendocrine carcinomas of the gut, pancreas, and lung [33]. Our results support these observations, since VAMP2, synaptophysin, syntaxin1, SNAP25, α/β-SNAP, and chromogranin A were detected in undifferentiated colorectal cancers. The same markers were also found in an associated adenocarcinoma (specimen no. 20) and in the normal mucosa. However, the extent of expression differed among the single markers: chromogranin A and VAMP2 were less frequently expressed. This is similar to an observation made by Schmitt-Graff et al. [33]. The synthesis of synaptophysin appears to be better maintained during neoplastic transformation [7, 33]. In our series of undifferentiated, neuroendocrine carcinomas, we found α/β-SNAP and synaptophysin to be expressed in all 9 tumors. SNAP25 was only expressed in 7 of 9 tumors. In addition, it was not only found at the plasma membrane as in normal neuroendocrine cells, but was also located in the cytoplasma, confirming the results of Schmitt-Graff et al. [33] (Fig. 1f, g). Since an intact syntaxin molecule is necessary to anchor SNAP25 to the plasma membrane [40], a disturbance in the regulated secretory pathway during neoplastic transformation seems likely.

Small cell undifferentiated neuroendocrine carcinoma of the colorectum carries the same abysmal prognosis as it does at other sites, and it clearly has important clinical implications. Such tumors, even when small and/or submucosal, are capable of rapid distant spread. The presence of even a small focus of such a tumor, in an adenoma for example, calls for radical surgery. The accumulated evidence is now sufficient to advocate multi-agent chemotherapy in all cases [21, 22, 27], since this tumor entity seems to be relatively chemosensitive [24]. In fact, the only ‘long-term-survivor’ in our study, a 57-year-old man with stage-IV disease and multiple liver metastases, received locoregional followed by systemic multi-agent chemotherapy.

Ongoing research in this area is necessary, because the better understanding of neuroendocrine differentiation in otherwise undifferentiated colorectal carcinomas could lead to innovative therapeutic strategies.

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