Small-cell neuroendocrine carcinomas (NECs) of the prostate are believed not to derive from benign orthotopic NE epithelial cells. Instead, an origin from a putative stem cell is actually the most favored concept. Whether this concept can also be applied to neuroendocrine tumors (NETs) of other organs, especially whether there are indications for well-differentiated NET–NEC sequence, is subject of the present study. A double-labeling technique for the proliferation marker MIB-1 and the NE markers chromogranin A (ChrA) and synaptophysin (SNP) was used for the immunohistochemical analysis of 45 well-differentiated NETs, 16 well-differentiated (low-grade) NECs, and 63 high-grade NECs of the esophagus, stomach, small intestine, appendix, colon, lung, prostate, and urinary bladder. The lowest proliferative activity was found in NETs (0.85% of tumor cells), and the highest activity was found in high-grade NECs (72.5%). The expression of ChrA was highest in NETs and lowest in high-grade NECs. None of the NETs and only sporadic cells in low-grade NECs showed double labeling (up to 0.05%). Up to 50% of the tumor cells in high-grade NECs were positive for MIB-1 and SNP. The percentage of double-labeled cells ranged between 0.9 and 39.6 (mean 9.7). No double-labeled cells were found in the normal epithelium adjacent to the tumors. Transitions from NET to NEC could not be observed. NETs and low-grade NECs differ in their proliferative activity from high-grade NECs, suggesting that they may arise from different precursor cell populations.

Keywords Neuroendocrine tumors · Neuroendocrine carcinoma · Low grade · High grade · Cell proliferation

Introduction

Our knowledge about the histogenesis of neuroendocrine tumors (NETs) is incomplete and includes theories that vary from organ to organ [3, 6, 10, 12, 18, 19, 20, 22]. NETs and well-differentiated neuroendocrine carcinomas (low-grade NECs) are believed by some authors to arise from orthotopic NE cells of the epithelium of the respective organs [6, 17, 18]. High-grade NECs (poorly differentiated) are believed not to derive from benign orthotopic neuroendocrine cells. Instead, an origin from a putative stem cell is currently the most favored concept [5, 13, 14]. This concept was studied in detail in carcinomas of the prostate with focal NE differentiation and in small cell NECs of the prostate [13, 14]. Whether NETs or low-grade NECs are able to transform into high-grade NECs (NET–NEC sequence) is unknown. If tumor groups can be separated from each other on the basis of their proliferative activity, they most likely represent different categories. If, however, the tumors proliferative activity overlaps, a differentiation into different groups appears to be unjustified. In order to test our hypothesis that NETs and low-grade NECs are different from high-grade NECs and probably derive from different cell sources, we have studied the proliferative activity of NETs and low- and high-grade NECs using the proliferation marker KI67/MIB-1 in combination with immunostaining for the NE markers chromogranin A (ChrA) and synaptophysin (SNP) [2, 5, 7, 8].

Methods

We studied 124 NETs classified according to Solcia et al. [23]. The tumors were localized to the esophagus (n=7), stomach (n=13), duodenum/small intestine (n=17), appendix (n=15), colon/rectum (n=8), pancreas (n=3), lungs (n=31), prostate (n=18), and urinary bladder (n=12; Table 1). After fixation in 4% buffered formalin and paraffin embedding, 4-µm-thick tissue sections were cut and stained for hematoxylin and eosin (HE) and for immunohistochemistry (IHC) analysis.

For the demonstration of NE differentiation, the markers ChrA (Camon; Wiesbaden, Germany; 1:1) and SNP (Biogenex; Hamburg, Germany; 1:100) were used. The proliferative activity was
evaluated with the MIB-1 antibody (Dianova; Hamburg, Germany; 1:50). Incubation time for all primary antibodies was 60 min. Before the application of MIB-1, slides were pretreated by means of microwave antigen retrieval (three times 600 W for 5 min). All IHC reactions were developed with the avidin–biotin-enhanced immunoperoxidase technique. Tonsillar tissue for Ki-67/MIB-1 and intestinal tissue for ChrA and SNP served as positive controls. The primary antibody was omitted for negative controls. In order to simultaneously analyze the proliferative activity and the expression of NE markers in tumor cells, we performed double stainings for ChrA and Ki-67/MIB-1. Ki67/MIB-1 staining was performed as described above, and ChrA was detected in a second staining sequence using the avidin–biotin complex method and 3-amino-9-ethylcarbazole (AEC; Dako; Hamburg, Germany) as a second chromogen. A case of malignant melanoma served as a positive control for this double-labeling method (MIB-1 and HMB 45).

All IHC-stained sections were assessed by one of us (B.H.). We analyzed 2000 cells from different areas of two histological slides for each immunostaining (in an area of highest staining intensity). The nuclear Ki67/MIB-1 labeling index (LI) was expressed as percentage of labeled cells. The staining intensity of ChrA/SNP was classified into four groups: 1–25% (+), 25–50% (++); 50–75% (+++); and 75–100% (+++). The number of Ki67 and ChrA double-positive cells was expressed as percentage of (1) all MIB-1-labeled cells and (2) as percentage of all ChrA positive cells. The differences of percentages of expressed NETs and NECs low/high-grade tumors were analyzed using the Student’s t-test.

Results

Well-differentiated NETs of the appendix measured less than 2 cm (range 0.8–2.6 cm, mean 1.4 cm) without extension into the mesoappendix. Two NETs measured 1.5 cm and 2.6 cm in diameter, with extension into the mesoappendix. The mean size of NETs from the other organs (stomach, ileum, and rectum) was 0.8 cm (range 0.1–1.0 cm) without angioinvasion. Low-grade NECs were predominantly localized in the ileum. Of 12 NECs, six measured 1.2–1.5 cm (mean 1.3 cm). The size of the other six tumors ranged from 2.9 cm to 9.0 cm (mean 3.95 cm). Of 12 low-grade NECs, five metastasized to the regional lymph nodes (n=3) and to the liver (n=2).

High-grade NECs were diagnosed in needle biopsy tissue of the lung, prostate, urinary bladder, pancreas, and liver. Biopsy material was collected from the esophagus and stomach. Complete tumors measuring 5–6 cm were diagnosed after resection of the stomach, the colon, the rectum, and the ovaries. The survival time of patients with high-grade NECs of the lung, the prostate, and the urinary bladder ranged between 5.5 months and 5.7 months. The death rate [death on disease (dod)] in patients with those high-grade NECs ranged between 60% and 70% (mean 62.5%).

Orthotopic epithelial NE cells of the prostate, stomach, small intestine, and appendix

ChrA/SNP-positive cells of the normal epithelium of the stomach, small intestine, and appendix adjacent to the tumor and of benign prostatic glands adjacent to the tumor were negative for MIB-1.

Well-differentiated NETs (carcinoids)

All NETs were strongly positive for ChrA/SNP (75–100%). The MIB-1 LI was very low (range 0.5–0.95%, minimum 0.1, maximum 2.0%). There was no significant difference between the mean MIB-1 LI of the NETs from different organs. Double-labeled cells with ChrA and MIB-1 were not demonstrable (Fig. 1, Table 1, and Table 2).

Well-differentiated NECs (low-grade)

Relative to the expression pattern in NETs, low-grade NECs showed a less homogeneous ChrA expression pattern (Fig. 2a), whereas there was no difference in the in-