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CASE REPORT

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Primary neuroendocrine carcinoma with ganglion cell differentiation in a crural lymph node

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Abstract A primary neuroendocrine carcinoma with ganglion cell differentiation is described in a crural lymph node. The patient, a 48-year-old woman, presented a palpable lymph node of the crural region in March 1994. Histologically, the lesion was composed mostly of small cells immunoreactive for cytokeratins, neuron specific enolase and synaptophysin. The small cells merged gradually with areas containing ganglion cells immersed in a fibrillar matrix resembling neuropil. Ganglion cells expressed neuron-specific enolase, synaptophysin, neurofilament proteins and S-100 protein. Moreover, a minority of them featured cytokeratin expression. Electron microscopy was performed in the small cell component. These cells featured attenuated desmosomes and electron dense granules with an average size of 120 nm within bundles of intermediate filaments. Clinically, no tumour was found elsewhere despite extensive work-up over the 76-month follow-up period. Although similarities with previous cases of primary neuroendocrine carcinoma of lymph node, ganglion cell differentiation has never been described.

Keywords Neuroendocrine carcinoma · Lymph node · Ganglion cell

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Introduction

Primary neuroendocrine carcinoma (NEC) of the lymph node is rare [7, 8]. The ten reported examples occurred in adults – six women and four men – aged between 37 years and 80 years (mean 57 years, median 64 years). Inguinal lymph nodes were the most common site. None of the patients were diagnosed with primary extranodal tumour at the time of clinical onset and extranodal neoplasm did not develop during the follow-up period. Eight patients were alive after a period ranging from 6 months to 70 months [7, 8]. Eusebi et al. suggested that NEC might originate from pre-existing intranodal epithelial nests or from a subset of nodal lymphoreticular cells expressing cytokeratins [7]. Despite some controversy [2, 3, 14], primary NEC is now accepted as a separate clinico-pathological entity. Herein, we describe a primary NEC of crural lymph node, featuring a unique ganglionic differentiation. No extranodal tumour was found 76 months after surgery.

Case history

The patient was a 48-year-old healthy woman. In March 1994, she underwent dissection of a palpable, painless left crural lymph node, which had been present for 1 year. Her familial and medical history was unremarkable. Laboratory findings were normal. No radio-chemotherapy was administered because clinical work-up failed to reveal any primary extranodal lesion. Work-up comprised of abdominal ultrasonography, total body computed tomography and bone scintigraphy. These investigations have been performed periodically since surgery. In June 2000, neither local recurrence nor extranodal tumour was found.

Materials and methods

Tissue was fixed in 10% buffered formalin and processed as routine. Sections (5 μm) were stained with routine haematoxylin-eosin and periodic acid–Schiff (PAS) stain with and without diastase digestion.

Immunohistochemistry

Using the avidin-biotin/peroxidase complex (ABC) method, immunohistochemical reactions were performed with antibodies directed against low-molecular-weight cytokeratins (Dako, Carpinteria Calif.; monoclonal MNF116, 1:200), cytokeratins 8, 18 (CAM5.2, Becton Dickinson, Calif.; monoclonal, prediluted), cytokeratin 20 (Dako; monoclonal KS10.8, 1:40), neuronal specific enolase (NSE; Biogenex, San Ramon, Calif.; monoclonal N3, 1:400), 68kDa and 200kDa neurofilament proteins (NF; Biogenex; monoclonal, NE14 1:30), S-100 protein (Dako; polyclonal 1:1500), chromogranin (Biogenex; monoclonal LK2H10, 1:200), glial acidic fibrillary protein (GFAP; Dako; polyclonal, 1:1000), vimentin (Dako; monoclonal V9, 1:50), p30/32mic2 (Dako; monoclonal 12E7, 1:50), desmin (Biomeda USA; polyclonal, 1:50), alpha smooth muscle actin (Dako; monoclonal 1A4, 1:100), sarcomeric actin (Dako; monoclonal alpha Sr-1, 1:20) and HMB45 (Dako; monoclonal HMB45, 1:50). Immunoreaction for synaptophysin (Biogenex; monoclonal SY38, 1:40) was performed using an avidin–biotin complex conjugated with alkaline phosphatase (Biogenex; San Ramon, USA) and developed with NBT/X-phosphate according to the manufacturer's instructions.

Electron microscopy

Samples for electron microscopy were recovered from paraffin-embedded material, post-fixed in 1% osmium tetroxide, dehydrated to absolute alcohol and embedded in araldite. Thin sections were counterstained with uranyl acetate and lead citrate and observed under a Philips 400T transmission electron microscope.

Results

Pathological findings

The lymph node measured 6 cm in its greatest axis. The cut surface was white with focal haemorrhages.

Histological findings

Large regions of the lymph node were involved by the tumour. A focal infiltration of the capsule was noted, whereas no neoplastic cell was seen within the marginal sinus. Most of the tumour featured incomplete lobules delimited by a thin framework of connective tissue rich in vessels. Lobules were composed of small polygonal cells showing pale cytoplasm. Nuclei were round and ovoid with coarse chromatin and a small eosinophilic nucleolus (Fig. 1). These elements gradually merged with areas constituted by large cells immersed in a mesh of fibrillary material resembling neuropil (Fig. 2). Large cells featured eosinophilic cytoplasm with peripheral deposition of granular amphophilic material reminiscent of Nissl's substance and showed ovoid vesicular nuclei with a prominent nucleolus (Fig. 3). In merging areas, there were neoplastic elements with intermediate features between the small and large cells. This gave the impression of a transition between the two components. Punctate necrosis, mitoses (10×10 HPF, ×400 using a Zeiss Plan-Neofluar 40/0.75 mm objective) and apoptotic bodies were present throughout the small cell component but were lacking in the large cell one. Calcification was absent. No cytoplasmic glycogen deposition was seen using PAS stain with and without diastase digestion.

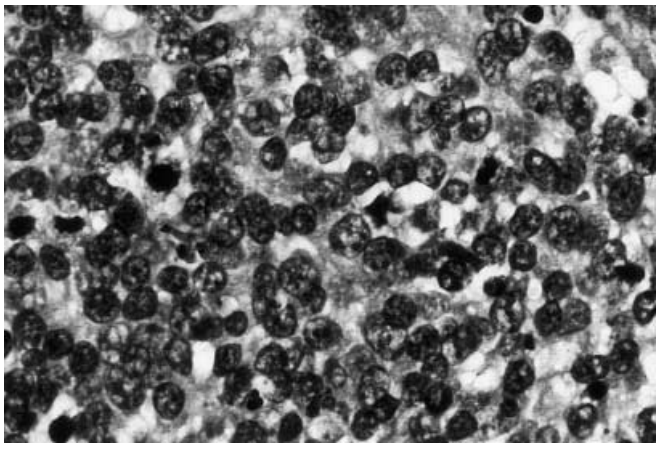

Fig. 1 Small cells feature scant cytoplasm and round to ovoid hyperchromatic nuclei with coarse chromatin and small nucleoli (haematoxylin and eosin, ×40)

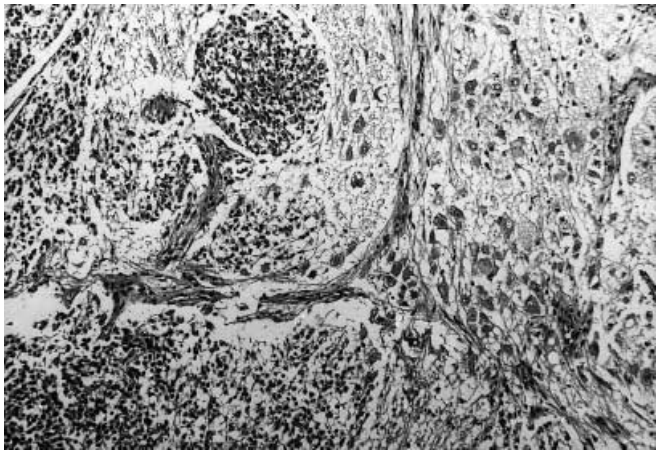

Fig. 2 The small cells gradually merge with areas constituted by ganglion cells immersed in a mesh of fibrillary material (haematoxylin and eosin, ×40)

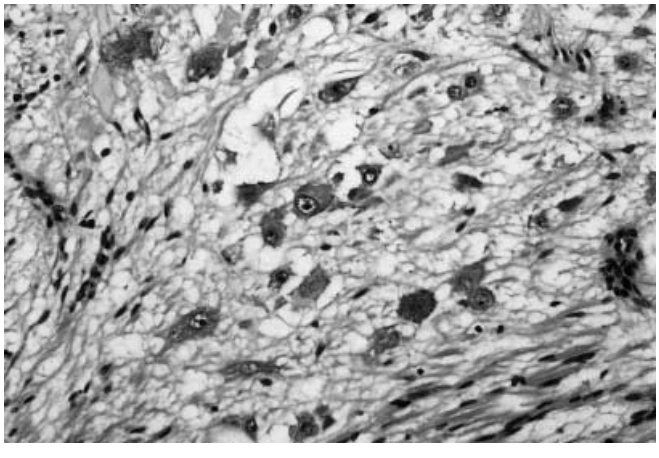

Fig. 3 Ganglion cells feature eosinophilic cytoplasm with peripheral deposition of granular amphophilic material and large, ovoid nuclei with prominent nucleoli (haematoxylin and eosin, ×25)

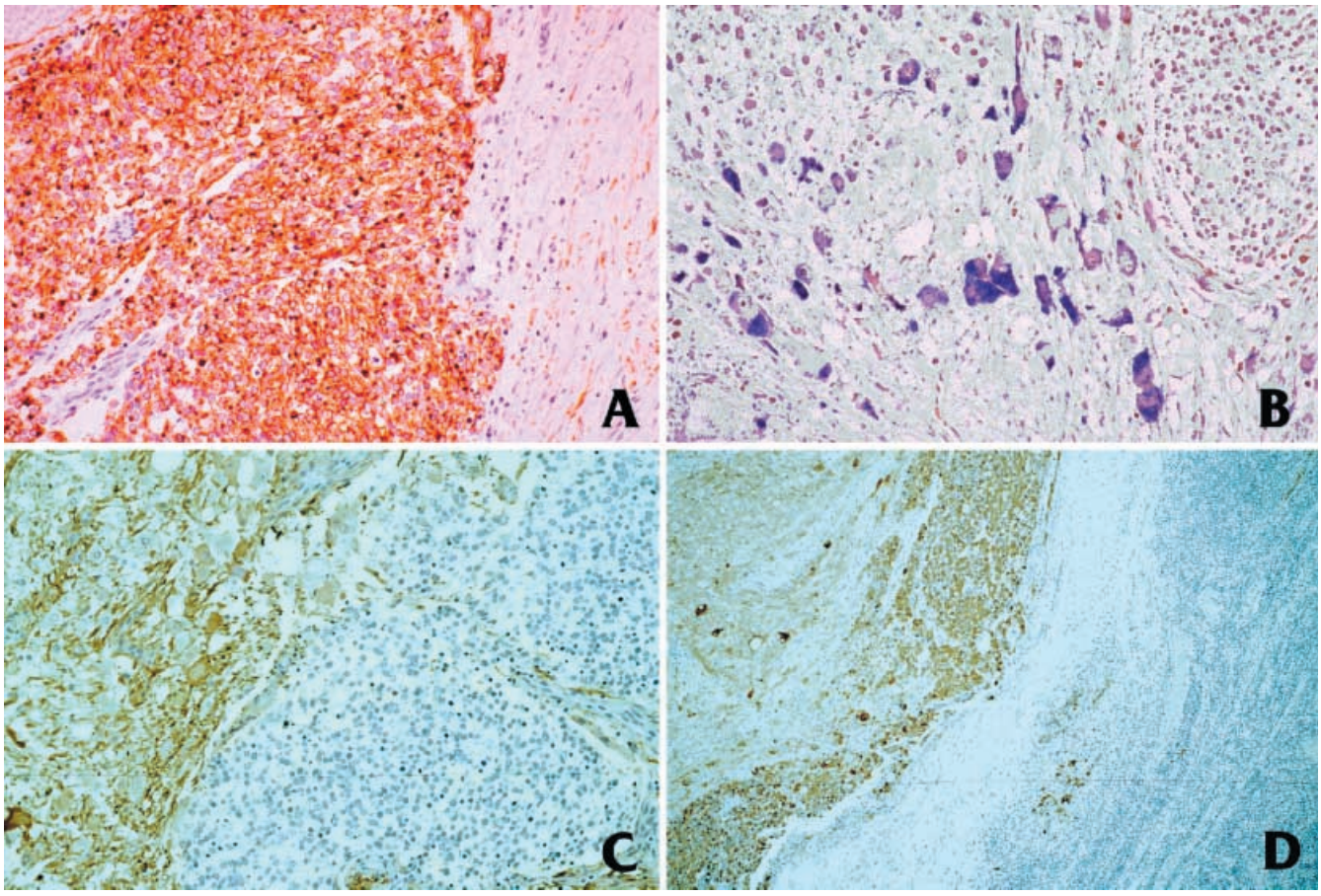

Fig. 4 **A** Small cells show a paranuclear dot-like reaction with antibody directed against low molecular weight cytokeratins [avidin-biotin/peroxidase complex (ABC)/peroxidase, ×10]. **B** Ganglion cells express synaptophysin (ABC/alkaline phosphatase, ×10). **C** Ganglion cells show neurofilament protein (ABC/peroxidase, ×10). **D** Low molecular weight cytokeratins are noted in some ganglion cells. Residual lymph node is also evident (ABC/peroxidase, ×4)

Immunocytochemistry

The small cells featured a paranuclear dot-like reaction with the antibody directed against low molecular weight cytokeratins (Fig. 4A). Cytokeratin 20 was noted in less than 5% of the cells. Small cells also reacted for NSE, synaptophysin and weakly for S-100 protein. Large cells were positive for NSE, synaptophysin (Fig. 4B), S-100 protein and neurofilament proteins (Fig. 4C). In addition, occasional elements contained low molecular weight cytokeratins (Fig. 4D). Fibrillary material stained with vimentin and neurofilament proteins, whereas synaptophysin was not detectable. The other antibodies gave a negative reaction.

Electron microscopy

Electron microscopy was performed in the small cell component, areas containing large cells being not available. The small cells featured round nuclei with dispersed chromatin and small-marginated compact nucleoli. The cytoplasm contained paranuclear whorls of loosely packed intermediate filaments containing sparse electron dense granules ranging in size from 100 nm to 180 nm (average 120 nm; Fig. 5a). Attenuated desmosomes were also noted (Fig. 5b). No neuritic process or microtubules were seen.

Discussion

We reported a primary lymph nodal NEC showing a unique ganglion cell differentiation. The tumour was composed mostly of epithelial cells resembling those of pulmonary well-differentiated NEC [15]. These elements featured para-nuclear dot-like immunoreactivity for cytokeratins and neuronal markers. Electron microscopy documented cytoplasmic whorls of intermediate filaments containing sparse electron-dense granules ranging between 100 nm to 180 nm in size. These are also features of NEC [6]. Merging with the small cells, there were large elements immersed in a fibrillary material. Their immunophenotype was that of ganglion cells, because they expressed synaptophysin, NSE and neurofilament proteins [9]. Moreover, like neuropil, fibrillary material stained for vimentin and neurofilament proteins. To our knowledge, no previous NEC with ganglionic differentiation has been described.

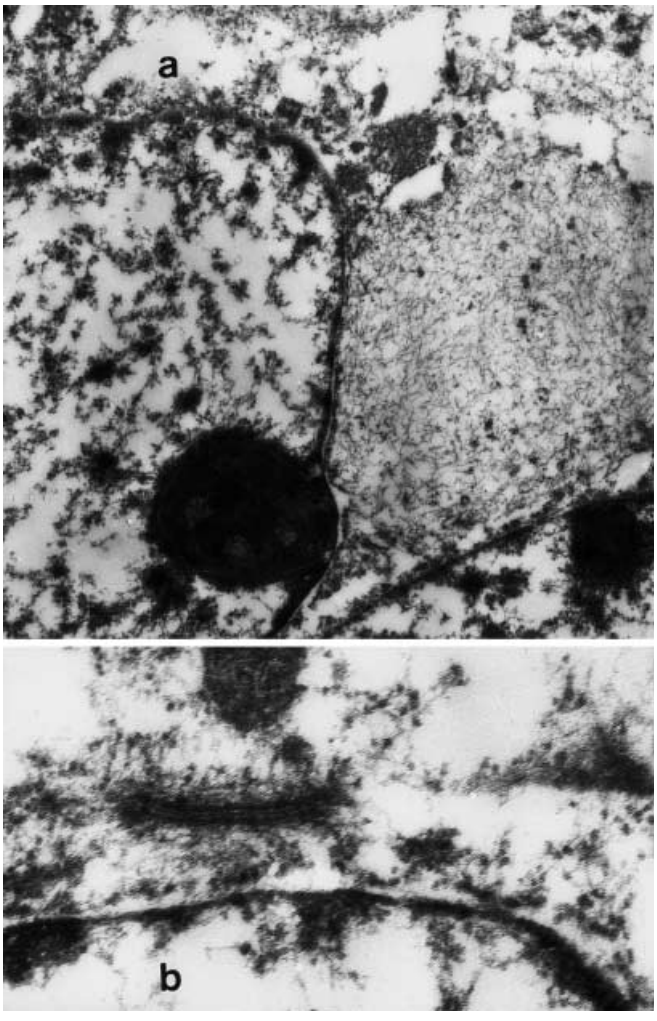

Fig. 5 Small cells show rounded nuclei with dispersed chromatin and small compact nucleoli. Cytoplasm shows paranuclear whorls of loosely packed intermediate filaments containing sparse electron dense neuroendocrine granules ranging in size from 100 nm to 180 nm (**a**; ×17). Attenuated desmosomes are present (**b**; ×66)

Intriguing but not surprising was the expression of low-molecular-weight cytokeratins seen in a minority of ganglion cells. It is of note that cytokeratin filaments were observed in the somata and processes of human embryonic cranial sensory neurons of the olfactory nerve, semilunar and vestibulo-cochlear ganglia, and in neurons migrating toward the brain along the olfactory nerve [13]. Moreover, cytokeratins 8 and 18 have been reported to be expressed in metaplastic neurons constituting pituitary adenoma with neuronal choristoma [10].

Similarly to human mid-gut carcinoid cells, which can be converted in vitro to neuron-like elements under stimulation of nerve growth factor [1], we interpreted ganglion cells as deriving from the neuroendocrine component. A gradual transition between the two cell types together with expression of low-molecular-weight cytokeratins in a carcinomatous component and ganglion cells seemed to sustain this view. The possibility of a collision tumour composed of NEC and gangliocytoma appeared unlikely.

The tumour described here resembled ganglioneuroblastoma. However, unlike ganglioneuroblastoma, the tumour lacked distinct Homer-Wright's rosettes and showed cytokeratin immunoreactivity in both the small and ganglion cells [11]. Ultrastructurally, small cells of ganglioneuroblastoma lack desmosomes and contain few dense-core neurosecretory-type granules aligned along tangled neuritic processes [11, 16]. Gangliocytic paraganglioma is composed of clusters of epithelioid “chief” elements surrounded by sustentacular cells. Cytokeratins are expressed in chief cells but not in ganglion cells. Upon ultrastructural examination, paraganglioma does not reveal cell-to-cell junctions, as seen here [12]. Peripheral neuroepithelioma often features lobules composed of undifferentiated small cells, but unlike NEC, neoplastic cells contain glycogen, express vimentin and a surface glycoprotein product of the gene MIC2 [5], lack neuropil and rarely show neuronal or epithelial differentiation [17]. Short neuritic processes, microtubules, dense-core granules located along neuritic processes and variable amounts of glycogen are ultrastructural hallmarks of peripheral neuroepithelioma but were lacking in the present lesion [5]. A case of metastatic melanoma with distinct ganglioneuroblastic differentiation has recently been described in an inguinal lymph node. Unlike the present tumour, neoplastic cells featured expression of S-100 protein and HMB-45 and lacked cytokeratins [4].

The lesion described here was considered a primary tumour of lymph node because neither extranodal neoplasm nor direct extension from a NEC of the overlying skin has been found over the follow-up period. Despite histologic features of high-grade tumour noted in the small cell component, the lesion showed an indolent behaviour. The patient is alive without local recurrence or metastatic dissemination 76 months after surgery. Ten cases of primary NEC of lymph nodes have been previously published. Their slides were provided by the authors [7, 8] and reviewed for this study. Histologically, all but two lesions closely resembled Merkel cell carcinoma, whereas the present and two previous tumours showed hyperchromatic nuclei with coarse chromatin and eosinophilic nucleoli, more similar to those of pulmonary well-differentiated NEC. No previous tumour disclosed ganglion cell differentiation. All lesions showed the dot-like paranuclear expression of low molecular weight cytokeratins noted in the present lesion and immunoreactivity for NSE and synaptophysin. The reactivity for cytokeratin 20, noted in a minority of neoplastic small cells in the present lesion, was too limited to warrant comments on a possible differentiation to Merkel cell carcinoma. Ultrastructural features were common in that neoplastic cells showed cytoplasmic paranuclear aggregates of intermediate filaments, endocrine granules whose average size was 130 nm and occasional desmosomes and intermediate junctions [7]. As seen in the present case, previous tumours had low malignant potential. After a follow-up period ranging from 6 months to 70 months, one patient was alive without evidence of disease, five had tumour relapse to adjacent or distant lymph nodes but were alive and well and two were alive with disease [7, 8].

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