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Case study

Primary gastric Merkel cell carcinoma harboring DNA polyomavirus. First description of an unusual high-grade neuroendocrine carcinoma

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Summary Merkel cell carcinoma (MCC) is a skin cancer that can also rarely arise in extracutaneous sites including mucosal surfaces. About 80% of MCCs harbor the Merkel cell polyomavirus (MCPyV). All cases of gastric MCCs so far reported were metastases from cutaneous sources. In the present article, we describe for the first time a primary gastric MCC harboring MCPyV. A 72-year-old man presented to clinical observation due to epigastric pain. Upper endoscopy revealed an ulcerated gastric tumor. The patient underwent total gastrectomy. The tumor was composed of mitotically active monomorphic small cells showing round nuclei with finely dispersed chromatin arranged in sheets and nests with large areas of necrosis. Tumor cells were positive for neuroendocrine markers and showed paranuclear dot immunoreactivity for cytokeratin 20. MCPyV was demonstrated with immunohistochemistry and electron microscopy, which showed intranuclear and intracytoplasmic viral particles. The MCPyV DNA in tumor cells was demonstrated with polymerase chain reaction analysis.

1. Introduction

Merkel cell carcinoma (MCC) is a relatively rare and aggressive skin cancer that can also arise in extracutaneous sites and can potentially metastasize to all organs of the human body [1]. Exceptional primary MCCs arising in mucosal surfaces have been described, but cases of gastric MCC so far reported in the literature were metastases from cutaneous sources. In the present article, we describe a

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primary MCC of the stomach harboring Merkel cell polyomavirus (MCPyV), which has been recently detected also in other extracutaneous MCCs [7].

2. Materials and methods

2.1. Case history

A 72-year-old man with a long history of chronic obstructive pulmonary disease and hypertension presented to clinical observation due to recent onset of increasing epigastric pain and 20-lb weight loss over the previous month. On examination, the patient was normotensive, and pallor of the conjunctiva and mild epigastric tenderness were seen. Laboratory results were significant for hemoglobin level of 8.5 g/dL and hematocrit of 28.1. Upper endoscopy revealed a large ulcerated mass extending from the cardias to the body of the stomach. A biopsy was performed, and histologic examination revealed sheets and groups of small lymphocyte-like neoplastic cells immunoreactive for pan-cytokeratin and chromogranin A consistent with a poorly differentiated neuroendocrine carcinoma. The patient underwent total gastrectomy and splenectomy. After the postoperative pathological diagnosis, the patient, questioned about history of cutaneous lesions, denied the presence of cancerous lesions of the skin. A complete and accurate examination of his skin showed no suspicious lesions.

A postoperative F^{18} fluorodeoxyglucose positron emission tomography performed 2 months later showed accumulation of the tracer in the right external iliac fossa, near the urinary bladder, consistent with a bulky metastatic lymph node or with a peritoneal neoplastic localization. An abdominal computed tomographic scan confirmed the presence of the metastatic lymphoadenopathy in the right iliac fossa. Salvage chemotherapy based on etoposide and cisplatin was initiated, but the patient died 2 months later. Autopsy was not performed.

2.2. Histology, immunohistochemistry, and electron microscopy

Routine hematoxylin and eosin–stained sections were prepared using a standard technique from formalin-fixed and paraffin-embedded (FFPE) blocks. Immunohistochemical analyses, including a panel of monoclonal and polyclonal antibodies (Table), was performed on 3-μm-thick sections cut from selected paraffin blocks using the Ventana Medical System Benchmark XT Ultra (Ventana, Tucson, AZ).

For electron microscopy, a piece of paraffin-embedded gastric MCC was used. The material was deparaffinized in chloroform, rehydrated, and washed in a 0.1 M sodium cacodylate buffer. Tissue fragments were postfixed in osmium and dehydrated and embedded in Epon-Araldite. Ultrathin sections contrasted with uranyl acetate and lead citrate were examined in a Morgagni electron microscope (Philips, Eindoven, the Netherlands).

Table Antibodies and antisera used

Antibodies/ Antisera	P/M (Clone)	Dilution	Source
Synaptophysin	M (snp88)	1:100	BioGenex Laboratories, San Ramon, CA
Chromogranin A	M (LK2H10)	1:1	Ventana
Cytokeratin 20	M ($K_5$20.8)	1:100	Dako, Carpinteria, CA
Cytokeratin 7	M (OV-TL 12/30)	1:200	Dako
TdT	P	1:1	Ventana
PAX 5	P	1:2000	Santa Cruz Biotechnology, Santa Cruz, CA
p63	M (4A4)	1:2	Cell Marque, Roklin, CA
Claudin 18	M (34H14L15)	1:300	Invitrogen, Carlsbad, CA
BCL2	M (124)	1:40	Dako
TTF1	M (8G7G3/1)	1:2	Ventana
PDX1	P	1:200	Santa Cruz Biotechnology
MCPyV	M (CM2B4)	1:100	Santa Cruz Biotechnology
p53	M (D07)	1:500	Dako
CD117	P	1:100	Dako
Ki-67	M (MIB1)	1:100	Dako

Abbreviation: P/M, polyclonal/monoclonal.

2.3. Polyomavirus DNA detection

Total DNA was extracted from FFPE tissue sections using the Qiagen Tissue Kit (Qiagen, Valencia, Spain) according to the manufacturer's instructions. DNA concentration and purity were determined by spectrometry. The maximum length of amplifiable DNA was assessed using multiplex polymerase chain reaction (PCR) on fragments of housekeeping genes of various sizes (100, 200, 300, 400, and 600 base pairs [bp]). The presence of MCPyV DNA was investigated using a PCR assay amplifying a 177-bp fragment targeted on the *T-Ag* gene [8]. Paraffin sections of nonneoplastic gastric tissue were used as a negative control, whereas DNA from a case of cutaneous MCC was used as a positive control.

2.4. Fluorescence in situ hybridization

Fluorescence in situ hybridization (FISH) analysis was performed on FFPE sections following the method previously reported [9]. Split-signal probes for *MYC*, provided by