Virchows Arch (1996) 427:537–541

CASE REPORT

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Ectopic adrenocorticotropin syndrome associated with undifferentiated carcinoma of the colon showing multidirectional neuroendocrine, exocrine, and squamous differentiation

Received: 30 June 1995 / Accepted: 14 November 1995

Abstract We report a rare case of ectopic ACTH syndrome associated with undifferentiated carcinoma of the ascending colon. A 62-year-old woman developed hypokalaemia and metabolic alkalosis associated with markedly elevated serum cortisol and plasma ACTH levels. High-dose dexamethasone (8 mg/day) did not suppress increased urinary 17-hydroxycorticosteroid and 17-ketosteroid excretion. Barium enema and abdominal computerised tomography showed a Borrmann II type tumour in the ascending colon, multiple metastatic nodules in the liver and bilateral enlargement of the adrenal glands. Histological examination of the resected primary colon cancer and metastatic liver tumour showed undifferentiated carcinoma with areas of distinct neuroendocrine, exocrine, and squamous differentiation. ACTH production by the tumour was confirmed by radioimmunoassay and immunohistochemistry. This is a unique case report of carcinoma of the colon with distinct multidirectional differentiation causing ectopic ACTH syndrome.

Key words Ectopic ACTH syndrome · Colon cancer · Multidirectional differentiation · Undifferentiated carcinoma

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Introduction

Ectopic adrenocorticotrophic hormone (ACTH) production is occasionally observed in various neoplasms, such as small cell carcinoma of the lung [1], but ectopic ACTH production causing Cushing's syndrome is not common. The tumours usually associated with ectopic ACTH syndrome are foregut carcinoids, pancreatic islet cell tumours, thymic epithelial carcinomas, medullary carcinoma of the thyroid gland, and pheochromocytoma. However, there have been few reports of ectopic ACTH syndrome associated with carcinoma of the colon [2–7]. We report a case of undifferentiated carcinoma of the colon causing this syndrome and review the relevant literature. Our case showed distinct areas of neuroendocrine, exocrine, and squamous differentiation.

Clinical history

A 62-year-old woman was admitted with malaise and weight gain of 5 kg in 1 month. On physical examination, mild pigmentation of the face and moderate pretibial pitting oedema were noted along with an elevated blood pressure (176/90 mmHg). She looked apathetic and depressed. However, there were no typical signs of Cushing's syndrome, such as moonface, buffalo hump, acne, or striae. Laboratory findings showed neutrophilia (14.3×10^9/l) and metabolic alkalosis (pH 7.55, HCO_3^- 42.9 mM) with hypokalaemia (serum K 2.0 mEq/l) due to excessive urinary excretion of potassium. Other biochemical data were normal except for hypoalbuminaemia (3.2 g/dl) and an elevated lactic dehydrogenase level (683 IU/l; normal 184–460 IU/l). Faecal occult blood was strongly positive.

Plasma renin activity was suppressed (0.1 $ngml^{-1}h^{-1}$), with a normal aldosterone concentration (99 pg/ml). The serum cortisol level was markedly elevated (138 μg/dl) and plasma ACTH (1400 pg/ml) was also raised and the normal diurnal variation of ACTH and cortisol was absent. Urinary excretion of 17-hydroxycorticosteroid (17-OHCS) and 17-ketosteroid (17-KS) was also increased to 61.7 mg/day and 24.6 mg/day, respectively. However, urinary excretion of 5-hydroxyindole acetic acid (5-HIAA) was normal (3.0 mg/day). Administration of high-dose dexamethasone (8 mg/day for 2 days) did not suppress the plasma ACTH level (1300 pg/ml before and after), the serum cortisol level (from 117 to 126 μg/dl), or the urinary 17-OHCS level (from 48.8 to 62.8 mg/day).

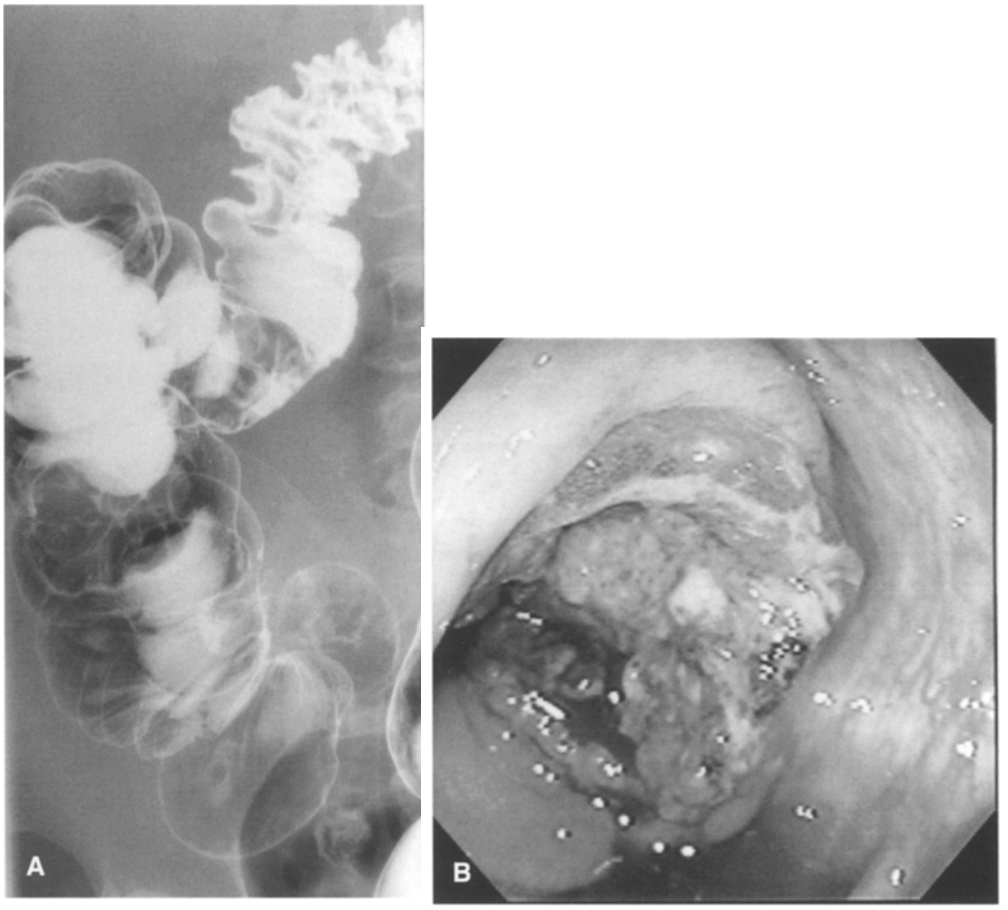

Fig. 1 **A** Barium enema shows a Borrmann II type colon cancer occupying around half of the lumen of the ascending colon. **B** Colonoscopy shows a round tumour with ulceration and bleeding

Thyroid function tests showed low triiodothyronine (T_3), thyroxine (T_4), and thyroid-stimulating hormone (TSH) levels (T_3, 52.0 ng/dl; T_4, 5.2 μg/dl; TSH, 0.22 μU/ml). The 75 g oral glucose tolerance test showed impaired glucose tolerance associated with a poor insulin response. Brain magnetic resonance imaging showed no abnormalities in the sellar region.

Computerised tomography of the abdomen demonstrated multiple nodules in the liver, with a huge mass in S5–6 and bilateral enlargement of the adrenal glands. A Borrmann II type colon cancer was detected in the ascending colon by barium enema (Fig. 1A) and colonoscopy (Fig. 1B) and was initially recorded as a poorly differentiated adenocarcinoma on biopsy. Tumour markers such as serum carcinoembryonic antigen, CA19-9, and alpha fetoprotein were within normal limits, but there was slight elevation of neuron-specific enolase (25.8 ng/ml; normal <10 ng/ml).

The patient was treated with parenteral potassium chloride supplementation (up to 170 mEq/day) and was also given metyrapone (750–2250 mg daily). Administration of metyrapone (750 mg) decreased the serum cortisol level (from 126 to 33.8 μg/dl) and did not increase the plasma ACTH level (from 1300 to 1200 pg/ml). Angiography of the coeliac trunk and superior mesenteric artery was performed on day 21 in hospital, and anticancer agents (epirubicin 10 mg, mitomycin C 4 mg, and lipiodol 3 ml) were infused into the right hepatic artery. The plasma ACTH level subsequently decreased to 750 pg/ml.

The ascending colon containing the primary tumour was resected from the terminal ileum to the hepatic flexure, and end-to-end anastomosis was performed to prevent bowel obstruction on day 41 in hospital. Wedge resection of the liver tumour was also done to determine whether the nodules were metastatic colon cancer. The plasma ACTH level was 830 pg/ml and the cortisol level was 9.4 μg/dl during metyrapone treatment (2250 mg/day) at 2 weeks after the operation. Her liver function gradually worsened and she died on hospital day 64. Autopsy was not permitted.

Materials and methods

Tissue ACTH concentration

Tissue ACTH was extracted with 1 mmol/l HCl and was measured by radioimmunoassay as described previously [8].

Histological studies

The primary tumour in the ascending colon and the resected liver lesion were fixed routinely in 10% buffered formalin and paraffin sections were stained with haematoxylin and eosin, PAS, and Grimelius' silver stain. Immunohistochemical staining by the avidin-biotin-peroxidase complex technique was also carried using paraffin sections and various monoclonal and polyclonal antibodies. The monoclonal antibodies used were CAM5.2 (prediluted; Becton-Dickinson, Mountain View, Calif.) for low-molecular-weight cytokeratin, 34βE12 (working dilution, 1:50; Dakopatts, Copenhagen, Denmark) for high-molecular-weight cytokeratin, and neuron-specific enolase (1:150; Dakopatts). We also used polyclonal antibodies directed against ACTH (1:1600; Dakopatts), somatostatin (1:400; Dakopatts), pancreatic polypeptide (1:500; IBL, Takasaki, Japan), serotonin (1:6000; Immuno Nuclear Corp., Stillwater, Minn.) to detect production of peptide hormones, and normal rabbit serum for negative control.

Results

The resected lesion in the ascending colon was a primary colon cancer and was mainly composed of undifferenti-

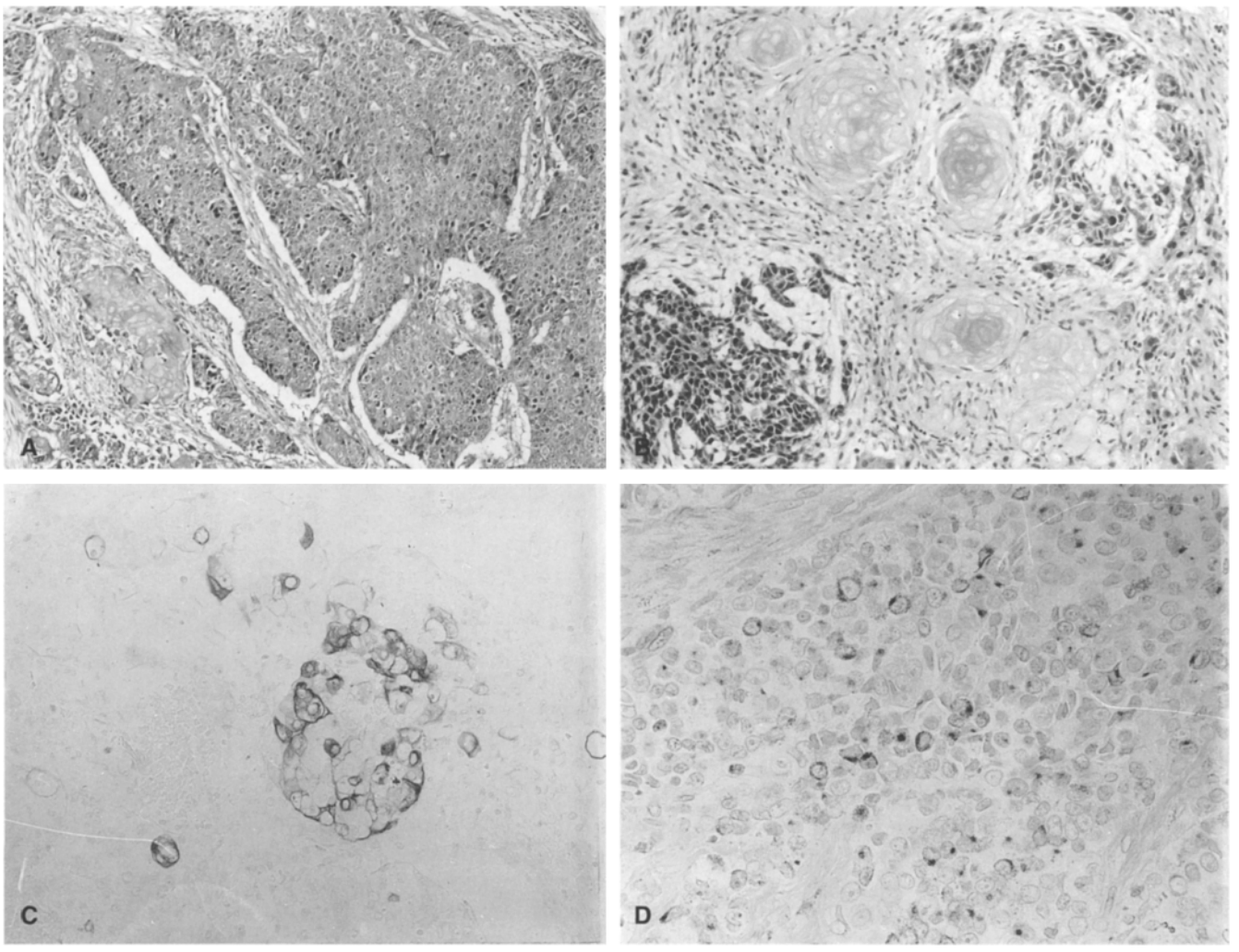

Fig. 2 **A** The primary colon cancer is chiefly undifferentiated carcinoma with focal glandular areas, and a squamous cell-like area is also present in the *left lower corner*. (HE, ×100). **B** Squamous differentiation is more evident in the metastatic liver tumour. (HE, ×150). **C** High-molecular-weight cytokeratin is positive in the cells with squamous cell differentiation in the primary colon cancer. (ABC method, ×200). **D** Many argyrophilic cells can be observed in the primary colon cancer. (Grimelius' stain, ×300)

ated intermediate-size cells, which were closely packed and arranged diffusely (Fig. 2A). However, some areas showed a glandular arrangement with PAS-positive mucin in the lumina, suggesting exocrine differentiation, and a squamous cell-like arrangement with distinct keratinization and cancer pearl formation was also observed (Fig. 2A). Many mitotic features (5–10/high-power view) and extensive necrotic areas were noted. Low-molecular-weight cytokeratin was immunohistochemically positive in all areas of the different features, while high-molecular-weight cytokeratin was focally positive in the cells showing squamous differentiation (Fig. 2C). Grimelius' silver staining revealed a positive reaction in many cells at several foci in the tumour (Fig. 2D). The number of these cells differed from area to area

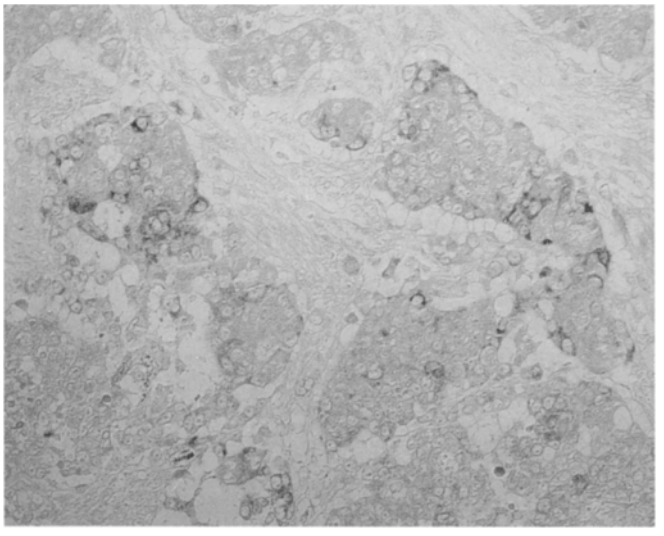

Fig. 3 ACTH immunoreactivity can be seen in some tumour cells in the primary colon cancer. (ABC method, ×200)

(1–20%). ACTH was positive in some tumour cells and in scattered small glandular lumina (Fig. 3). Somatostatin, pancreatic polypeptide, serotonin, and neuron-specific enolase were all negative. The metastatic liver tumour showed similar histological features, but the squamous differentiation and keratinization were more prominent (Fig. 2B). ACTH was positive, as in the colon tumour. The immunoreactive ACTH content of the primary colon tumour and the metastatic liver tumour was 30 and 19 ng/g wet weight tissue, respectively, while the ACTH content of adjacent normal colonic mucosa was less than 2 ng/g wet weight.

Discussion

The present patient showed severe hypokalaemia, metabolic alkalosis, water retention, and depression caused by an elevated cortisol level, and skin pigmentation also increased with the progression of her disease. The bilateral enlargement of the adrenal glands suggested the highly stimulated state of the glands by ectopic ACTH production leading to the massive cortisol secretion. The absence of typical signs of Cushing's syndrome, such as moonface, centripetal obesity, or striae, suggested rapid progression. Ectopic ACTH production by the tumour was confirmed by the results of ACTH immunohistochemistry and radioimmunoassay. Plasma ACTH levels decreased after transcatheter arterial chemotherapy, but were hardly influenced by resection of the primary colon cancer, suggesting that the metastatic liver nodules were the main source of ACTH production. The primary colon cancer contained a higher concentration of ACTH than the metastatic liver tumour. However, angiography showed that the liver metastasis was much larger than the primary tumour, and this probably contributed to the massive ACTH production.

There have been few reported cases of ectopic ACTH syndrome associated with carcinoma of the colon (Table 1) [2–7]. Disease progression was very rapid in all cases, and the patients died after a relatively short period (17 days to 12.5 months). The tumours were frequently accompanied by liver metastasis (6 out of 7 cases). Plasma ACTH and serum cortisol levels were markedly elevated in each case, with severe hypokalaemia (1.7–3.5 mEq/l), as in the present case. Histologically, all the tumours but one were undifferentiated or small cell carcinomas. Undifferentiated carcinoma of the colon and rectum was first reported by Clery et al. [9] as small cell carcinoma and comprises about 0.2–0.4% of all malignancies in this region. Gould and Chejfec [10] reported that these undifferentiated carcinomas had a neuroendocrine cell component, since they showed a positive Grimelius' reaction and positive immunohistochemical staining for neuron-specific enolase, and were found to have specific small round endocrine granules in the cytoplasm on electron microscopy. These undifferentiated carcinomas with a neuroendocrine component grow rapidly and produce widespread metastases, resulting in a very poor prognosis. However, ACTH production by neuroendocrine carcinoma of the colon is very rare [11]. Since a very small amount of ACTH messenger RNA is detected in normal colon [12] the mechanism of augmented ACTH expression in neuroendocrine carcinoma of the colon remains to be determined.

The tumour of our patient showed exocrine and squamous differentiation, in addition to components of undifferentiated carcinoma and nests of neuroendocrine cells. Damjanov et al. [13] reported a case of colon cancer with similar histology in which the tumour was composed predominantly of undifferentiated cells with foci of neuroendocrine, exocrine, and squamous cells, occasionally arranged in an organoid manner. Histochemically, the tumour contained argyrophilic cells as well as cells that reacted positively with antibodies to alpha-1-antitrypsin, alpha-1-antichymotrypsin, carcinoembryonic antigen, and lysozyme. They proposed that this type of tumour could be designated a "stem cell carcinoma". Petrelli et al. [14] also suggested that their case of colon cancer containing undifferentiated, carcinoid, and squa-

Table 1 Clinical characteristics of seven patients with ectopic ACTH syndrome associated with carcinoma of the colon (*ND* not done)

No.	Age / Sex	Site	ACTH/Cortisol (pg/ml)	ACTH/Cortisol (μg/dl)	Histology	Status	Reference
1	52 M	Ascending[a]	2.5[b]	49	Small cell Ca., poorly differentiated adenocarcinoma	Dead (17 days)	[2]
2	60 M	Sigmoid[a]	177	58	Moderately well differentiated adenocarcinoma	Dead (5 weeks)	[3]
3	45 F	Unknown[a]	ND	74.3	Adenocarcinoma	Dead	[4]
4	33 F	Unknown[a]	2340	ND	Undifferentiated Ca.	Dead (1 month)	[5]
5	74 M	Rectum	234	53.3	Small cell Ca.	Dead (12.5 months)	[6]
6	25 M	Anorectal[a]	1967	60	Poorly differentiated Ca., squamous cell Ca., small cell Ca.	Unknown	[7]
7	62 F	Ascending[a]	1400	138	Undifferentiated Ca., adenocarcinoma, squamous cell Ca., endocrine Ca.	Dead (2 months)	Present case

[a] With liver metastasis
[b] mU/dl

mous cells may have originated from a pluripotential stem cell. Recently, Staren et al. [11] demonstrated that 8 out of 13 neuroendocrine carcinomas of the colon and rectum had foci of glandular and/or squamous differentiation. It seems likely that the colon cancer of our patient originated from a neoplastic pluripotential stem cell and was capable of differentiating in multiple directions.

Acknowledgements The authors are grateful to Dr. Kazuhiko Yamagami, Department of Surgery, and Dr. Akira Furukawa, Department of Radiology, Saiseikai-Noe Hospital, Osaka for their support.

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