

Paraneoplastic REG1 α Cast Nephropathy Associated With Mixed Acinar-Neuroendocrine Carcinoma



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INTRODUCTION

Paraneoplastic syndrome refers to clinical manifestations of a malignancy that are not directly related to the tumoral burden or direct invasion but rather to substances secreted by the tumor, including peptides, cytokines, and tumor antigens.¹ Membranous nephropathy is the most common solid cancer-associated paraneoplastic kidney lesion, whereas myeloma cast nephropathy (MCN), amyloid light-chain amyloidosis, and minimal change disease are the most frequent kidney lesions associated with hematologic malignancies.¹ We present a rare case of paraneoplastic myeloma-like cast nephropathy associated with mixed acinar-neuroendocrine carcinoma (MANC). Proteomic and immunohistochemical (IHC) analysis revealed that the tubular casts were composed of regenerating protein 1 alpha (REG1 α).

CASE PRESENTATION

A 38-year-old man presented with a 2-month history of abdominal discomfort, early satiety, nausea, vomiting, and weight loss. His personal and family medical histories were unremarkable. He was found to have a serum creatinine of 299 μ mol/l, rising to 1044 μ mol/l 7 days later. Laboratory findings on admission are shown in Table 1. Serum protein electrophoresis with immunofixation did not show monoclonal protein.

On examination, he had ascites (5 l). An abdominal computed tomography scan revealed ascites and nodular studding of the peritoneum, with a thickened omentum and mesentery, consistent with peritoneal carcinomatosis. The kidneys showed no evidence of

obstruction or involvement by the tumor. A biopsy specimen of the omental tumor was obtained and was initially reported as an adenocarcinoid, but on subsequent review it was consistent with MANC of the pancreas.

The patient was started on hemodialysis. The tumor was inoperable, and chemotherapy was considered, but the patient died before additional therapy could be begun, 3 weeks after undergoing the biopsy procedure.

Autopsy Findings

An autopsy revealed an extensive tumor burden throughout the peritoneal cavity. The pancreas was completely infiltrated by the tumor, suggesting pancreatic origin. The bone marrow showed no excess of plasma cells, and there was no lymphadenopathy.

The pancreatic neoplasm was composed of uniform epithelioid cells with round nuclei and prominent nucleoli, arranged in solid nests with focal areas of pseudoglandular formation (Supplementary Figure S1). Frequent mitoses were present. IHC characterization showed diffuse tumor staining for synaptophysin, trypsin, carboxypeptidase A1 (CPA1), REG1 (Supplementary Figure S1), chromogranin, and pan keratin. The histology and IHC profile supported a pathologic diagnosis of MANC. Electron microscopy (EM) on tumor cells revealed zymogen granules consistent with acinar differentiation.

The kidneys displayed severe acute myeloma-like cast nephropathy. Numerous distal tubular casts were seen, which appeared fractured, hyper eosinophilic on hematoxylin and eosin stain, negative with periodic acid–Schiff stain, polychromatic on trichrome stain,

Table 1. Laboratory findings on admission

Parameter	Value (reference range)
Serum creatinine ($\mu\text{mol/l}$)	1008 (60–120)
Blood urea nitrogen (mmol/l)	28.5 (3.0–7.5)
Sodium (mmol/l)	127 (135–145)
Potassium (mmol/l)	6.0 (3.5–5.0)
Calcium (mmol/l)	2.15 (2.10–2.55)
Phosphate (mmol/l)	3.11 (0.80–1.50)
Serum albumin (g/l)	22 (35–50)
Hemoglobin (g/l)	130 (137–180)
Hematocrit	0.38 (0.40–0.54)
Platelets ($10^9/l$)	405 (150–400)
White blood cell count ($10^9/l$)	11.2 (4.0–11.0)
Neutrophils ($10^9/l$)	9.1 (2.0–8.0)
Serum protein electrophoresis with immunofixation	No M-spike

and negative on Congo red stain (Figure 1). They elicited a prominent multinucleated giant cell reaction. Similar casts were seen extravasated into the interstitium. There was acute tubular injury and mild interstitial inflammation, and only mild interstitial fibrosis. The casts were negative for kappa and lambda by immunofluorescence on paraffin tissue. On EM, the majority were uniformly composed of randomly oriented fibrils with a mean thickness of 13 nm (range 7–21 nm; Figure 1), with occasional peripheral spicules, while a minority showed granular texture of the center and fibrillar texture of the periphery. The casts exhibited strong and diffuse IHC staining for REG1 (Figure 2d), with only weak and focal staining for CPA1 and chromogranin, and negative staining for myoglobin and hemoglobin. Periodic acid–Schiff–negative protein droplets were seen in occasional parietal and tubular epithelial cells, which exhibited strong staining for REG1 and granular (nonfibrillar) texture on EM. Glomeruli were unremarkable.

Proteomic Analysis

A previously described procedure was used to characterize the proteomic content of the renal casts and tumor cells.² Casts were laser microdissected from a 10- μm -thick kidney section of paraffin-embedded necropsy specimen. Two independent dissections were collected, and the proteins present were processed and subjected to mass spectrometry analysis using a previously described protocol.³ Separately, tumor cells were microdissected and analyzed by mass spectrometry. Figure 2 shows the protein identification profile of casts. A large number of MS/MS spectra (average 911) were matched to REG1 α , suggesting an overabundance of this protein in the casts (Figure 2a). A much smaller number of spectra matched to REG1 β (average 43) and REG3 α (average 21; Figure 2a). No spectra for CPA1 or chromogranin A were detected. Small numbers of spectra for REG1 α were also detected in tumor cells

(data not shown). The sequence coverage maps (Figure 2b and 2c) show the portions of REG1 α and REG1 β that were detected by the mass spectrometry in the casts.

DISCUSSION

REG proteins are small secretory C type–like lectins that promote pancreatic islet growth in response to inflammation or injury. The human REG family consists of 5 family members: REG1 α , REG1 β , REG3 α , REG3 γ , and REG4, which share 30% to 80% sequence homology. REG1 α , also known as pancreatic stone protein and lithostathine 1 α , is a 144-amino acid protein predominantly expressed in pancreatic acinar cells. It is highly upregulated in acinar cell carcinoma (ACC) and MANC. The latter is defined by the presence of >30% of both acinar and neuroendocrine cell types by IHC. REG1 α serves as a useful diagnostic marker for acinar cell differentiation. We recently reported 84% and 93% sensitivity and specificity, respectively, of diffuse/patchy IHC staining for REG1 α in distinguishing ACC/MANC from other pancreatic tumors.⁴ However, ectopic expression, usually focal, may occur in other pancreatic tumors,⁴ gastric carcinoma,⁵ and intrahepatic carcinoma. REG1 α circulates in the blood at low levels in healthy individuals. Its serum level is markedly elevated in patients with cancer (particularly patients with gastrointestinal and metastatic cancers) and therefore it was proposed as potential biomarker in predicting cancer occurrence.⁶ Higher serum concentrations have also been reported in association with chronic obstructive pulmonary disease, sepsis, diabetes, and renal dysfunction in pregnant women.⁷

Two case reports describing irreversible acute kidney injury caused by myeloma-like cast nephropathy in association with ACC have been reported, although a pathogenetic link was not established.^{8,9} We recently reported a third case of myeloma-like cast nephropathy in association with MANC in which proteomic analysis and IHC identified 2 acinar cell–specific proteins, REG1 α and CPA1 in tubular casts and tumor cells.² In the patient described here, large numbers of protein spectra for REG1 α were detected in the atypical casts by proteomic analysis with strong staining for REG1 by IHC, consistent with paraneoplastic REG1 cast nephropathy.

The secretory form of REG1 α has highly aggregative properties under physiologic conditions, and it is prone to self- and trypsin-mediated proteolysis because of a specific cleavage of Arg¹¹-Ile¹² peptide bond, generating a 133-amino acid COOH terminal domain that precipitates as amyloid-like unbranched fibrils measuring 12 nm in diameter.^{S1,S2} By EM, the atypical casts in our patient were composed of amyloid-like fibrils, consistent

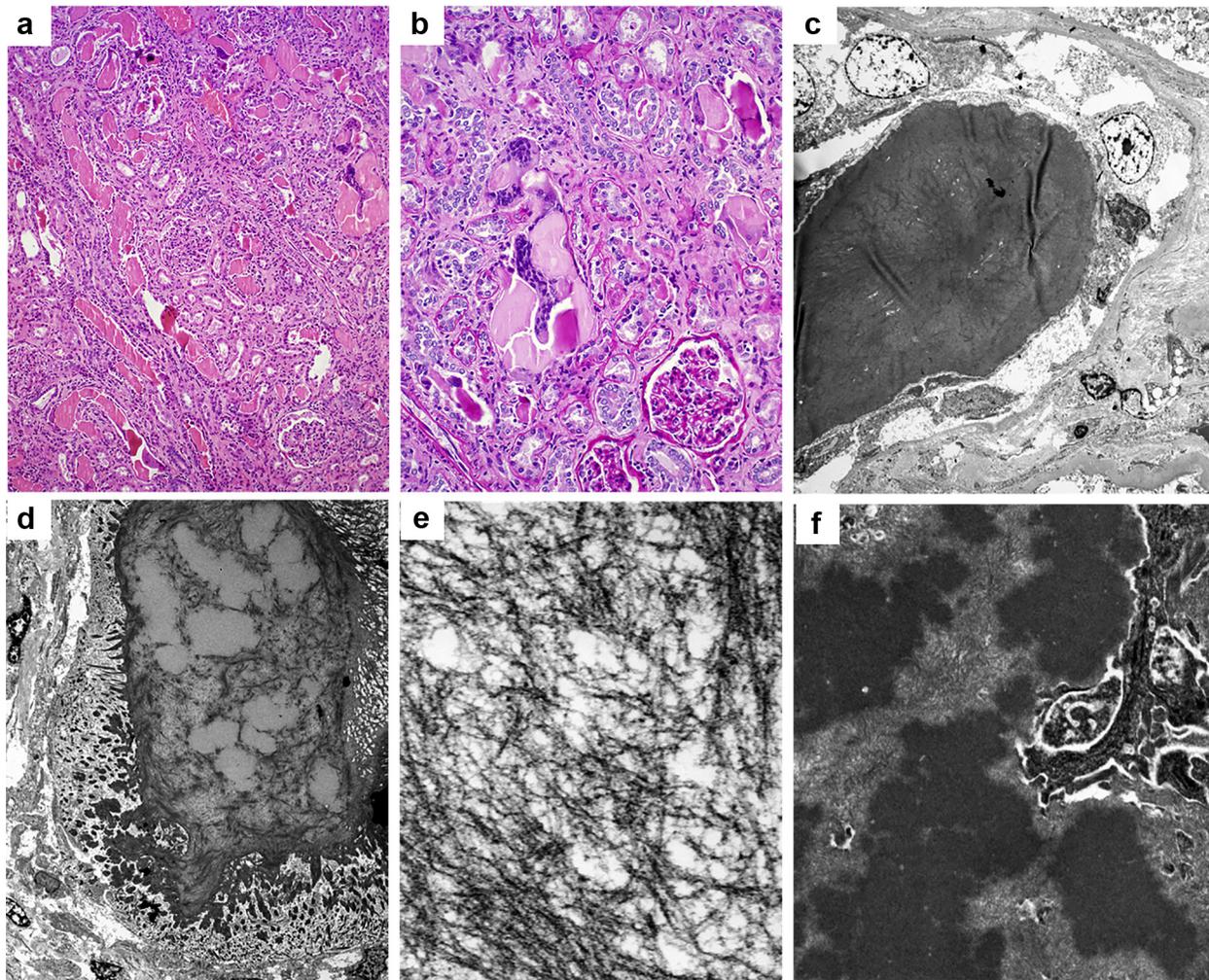


Figure 1. Pathology of cast nephropathy. (a) Low power image showing diffuse acute tubular injury and many large, hypereosinophilic, fractured distal tubular casts (hematoxylin and eosin stain; original magnification $\times 100$). (b) The atypical casts were negative on periodic acid–Schiff stain, some of which were associated with a giant cell reaction, reminiscent of myeloma casts (original magnification $\times 200$). (c) Low power electron microscopy image showing a large electron-dense cast obstructing the distal tubular lumina (original magnification $\times 2000$). (d) An atypical cast showing peripheral spicular projections, reminiscent of amyloidogenic cast nephropathy (original magnification $\times 2000$). (e) On high magnification, most atypical casts were uniformly composed of randomly oriented, nonbranching fibrils with a mean thickness of 13 nm ($\times 50,000$). (f) Few atypical casts showed granular texture of the center and fibrillar texture of the periphery (original magnification $\times 8000$).

with aggregated REG1 α protein. Furthermore, proteomic analysis of the casts revealed mostly peptides of the cleaved form of REG1 α (although our mass spectrometry methodology cannot distinguish REG1 self-proteolysis from trypsin digestion). We propose that cast nephropathy in our patient likely resulted from a secretion of massive amounts of REG1 α by carcinoma cells, which by virtue of its low molecular weight (19 kDa) freely filtered through the glomerular filtration barrier, overwhelmed proximal tubular reabsorption capacity, and precipitated in distal tubular lumina, leading to tubular obstruction, acute tubular injury, and interstitial inflammation. REG1 α fibrillogenesis likely occurred within tubular lumina as REG1 granules within proximal tubular and parietal epithelial cells were non-fibrillar. It remains to be determined if REG1 α causes

direct toxicity to tubular cells or interacts with Tamm–Horsfall protein in distal tubular lumen, similar to nephropathic light chains in MCN.

Our patient and the 3 previously reported patients with ACC/MANC-associated paraneoplastic cast nephropathy^{2,8,9} did not recover kidney function; therefore, renal prognosis appears to be dismal, reflecting the aggressive tumor behavior and poor patient outcome.

Obtaining a kidney biopsy specimen should be considered in patients with acute kidney injury and a recent diagnosis of pancreatic ACC, MANC, or neuroendocrine carcinoma (which can resemble MANC morphologically), to exclude paraneoplastic cast nephropathy. The light microscopic morphology of REG1 α cast nephropathy in the case presented here is

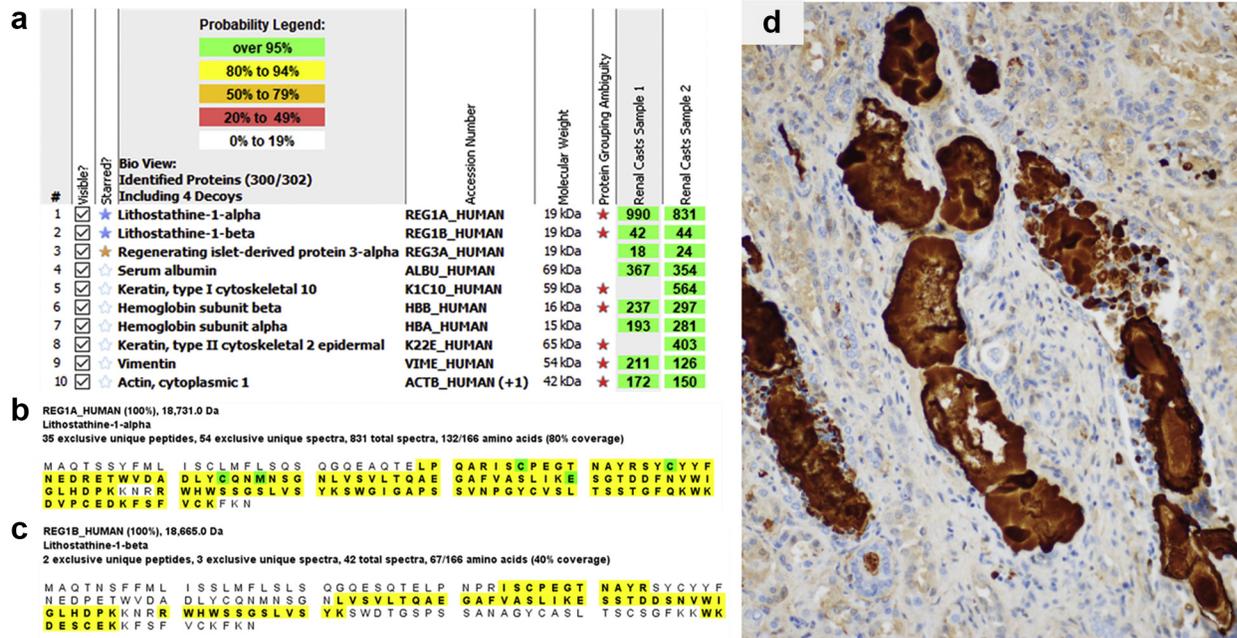


Figure 2. Proteomics and immunohistochemistry identify regenerating protein 1 (REG1) as the primary constituent of renal casts. (a) Renal casts were laser microdissected and subjected to mass spectrometry–based proteomics analysis. The protein identification profile obtained from independent microdissections is shown. Numbers in green boxes represent the number of mass spectrometry spectra matching to the protein in the respective sample (i.e., spectral counts). Spectral counts are a measure of a protein’s relative abundance in the deposit. Proteins with higher spectra counts are of higher abundance than proteins with lower spectral counts. The blue stars highlight the primary pathogenic protein REG1 α and REG1 β isoforms. The yellow star highlights REG3 α protein that is also native to the tumor cells and observed in the casts. (b and c) Sequence coverage of REG1 α and REG1 β in the samples. Yellow portions of the protein sequence were detected by mass spectrometry in the patient casts. Proteomics show that both α and β forms of REG1 are incorporated in the renal casts with REG1 α being the major component. (d) Tubular casts show intense immunohistochemical positivity for REG1 α (original magnification $\times 200$).

identical to MCN (Table 2). Other types of cast nephropathy that can mimic MCN include chromogranin cast nephropathy associated with neuroendocrine neoplasms,⁵³ drug-induced cast nephropathy, such as associated with rifampin,⁵⁴ rapamycin,⁵⁵ or vancomycin,⁵⁶ and myoglobin and hemoglobin cast nephropathies.⁵⁷ The distinction from MCN is based on the lack of light chain restriction of casts on immunofluorescence and the clinical history. Contrary to classic MCN, which typically exhibits a highly electron-dense granular or paracrystalline substructure, REG1 casts in this case were composed of randomly oriented amyloid-like fibrils. Therefore, the ultrastructural appearance

can overlap with amyloidogenic MCN,⁵⁸ but unlike the latter, REG1 casts are hyper eosinophilic on hematoxylin and eosin stain and negative on Congo red stain. Chromogranin casts were also reported to have a fibrillar texture, but contrary to REG1 casts, the fibrillary material is organized in parallel curvilinear bundles.⁵³ Tamm–Horsfall protein casts also have a fibrillar texture on EM but their ultrastructural appearance is different from amyloidogenic MCN and REG1 casts, and they are periodic acid–Schiff–positive and have a “frothy appearance” on light microscopy.^{57,59}

Table 2. Teaching points

- 1 Solid cancer–secreted proteins can be nephropathic by forming obstructive tubular casts, resulting in acute kidney injury
- 2 Acinar cell carcinoma/mixed acinar-neuroendocrine carcinoma should be considered in the differential diagnosis of cast nephropathy when immunofluorescence fails to show light chain restriction of the casts or in the absence of laboratory evidence of multiple myeloma
- 3 Obtaining a kidney biopsy specimen should be considered in patients with acute kidney injury and recent diagnosis of pancreatic acinar cell carcinoma/mixed acinar-neuroendocrine carcinoma or neuroendocrine carcinoma, to exclude paraneoplastic cast nephropathy
- 4 In patients with myeloma-like cast nephropathy, proteomic analysis is a useful tool to determine the composition of casts and may provide insights into its pathophysiology

CONCLUSION

This report provides supportive evidence that solid cancer–secreted proteins can cause acute kidney injury by forming obstructive distal tubular casts, a largely uncharacterized pathomechanism for carcinoma-associated nephropathy.

DISCLOSURE

This case discussed was part of a study on nonmyelomatous cast nephropathy, for which an institutional review board approval and waiver of

consent from the patient's family have been obtained. All the authors declared no competing interests.

SUPPLEMENTARY MATERIAL

Supplementary File (PDF)

Figure S1. Pathology of pancreatic tumor.

Supplemental References.

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