Pancreatic solitary fibrous tumor causing ectopic adrenocorticotropic hormone syndrome

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Article info

Article history:
Received 21 August 2016
Received in revised form 26 August 2016
Accepted 28 August 2016
Available online 29 August 2016

Keywords:
Pancreas
Solitary fibrous tumor
Ectopic adrenocorticotropic hormone syndrome
Proopiomelanocortin
Adrenocorticotropic hormone

Abstract

Solitary fibrous tumors occasionally present with hypoglycemia because of the excessive release of insulin-like growth factor II. We report the first case of pancreatic solitary fibrous tumor causing ectopic adrenocorticotropic hormone syndrome. An 82-year-old Japanese man presented with lower limb edema, uncontrolled hypertension, hypokalemia, and baseline hypercortisolism. Distal pancreatectomy was performed after the clinical diagnosis of a neuroendocrine tumor with ectopic secretion of adrenocorticotropic hormone. On histological examination, the tumor showed spindle cells in a fascicular arrangement. The diagnosis of the solitary fibrous tumor was confirmed by the identification of the NAB2-STAT6 fusion gene and positive immuno-histochemical staining for STAT6 and CD34. Using quantitative real-time polymerase chain reaction, mRNA that encoded proopiomelanocortin, precursor of adrenocorticotropic hormone, was detected. Proopiomelanocortin production through the demethylation of the promoter region Domain IV was detected. Pancreatic solitary fibrous tumors represent a new cause of ectopic adrenocorticotropic hormone syndrome.

1. Introduction

Solitary fibrous tumors (SFTs) are spindle cell tumors of mesenchymal origin. Although these tumors have been described in almost every anatomical location, the pancreas is an extremely rare site. It is well known that some patients with SFT present with hypoglycemia because of the excessive release of insulin-like growth factor II (IGF-II) (Hajdu et al., 2010). However, to the best of our knowledge, there is no case report of ectopic adrenocorticotropic hormone syndrome (EAS) secondary to SFT. EAS is a disorder of proopiomelanocortin (POMC) gene expression in non-pituitary tumors (Beuschlein and Hammer, 2002). In the present study, we report the first case of a male patient who presented with pancreatic SFT and also developed EAS.

2. Material and methods

2.1. Subject

An 82-year-old Japanese man was admitted to our hospital for the evaluation of lower limb edema, uncontrolled hypertension,
and hypokalemia. He underwent cholecystectomy for cholecystolithiasis 53 years ago. His family history was unremarkable. Physical examination revealed central obesity, lower limb edema, and a blood pressure of 176/100 mmHg despite treatment with anti-hypertensive drugs. Preoperative sodium and potassium levels were 138 mmol/L and 3.0 mmol/L, respectively. Baseline hypercortisolism was confirmed with a markedly increased urinary free cortisol of 1469 μg/day (reference range, 26–187 μg/day). The plasma adrenocorticotropic hormone (ACTH) concentration was elevated at 189 pg/ml (reference range, 7.2–63.3 pg/ml). Baseline serum cortisol level at early morning (0500 h) was elevated at 29.5 μg/dl (reference range, 4.0–23.3 μg/dl). The high dose dexamethasone suppression test (at 8 or 12 mg) slightly decreased the plasma cortisol level to 25.8 μg/dl (87.5%) or 24.0 μg/dl (81.4%), respectively from baseline. The corticotropin-releasing hormone test resulted in a 3.2% decrease (183 pg/ml) of the serum ACTH level from baseline. The contrast-enhanced computed tomography revealed a well-circumscribed tumor that was 6 cm in diameter with an inhomogeneous enhancement near the pancreatic tail (Fig. 1A). There was no defined adrenal tumor. Axial magnetic resonance images (MRI) revealed a tumor with relatively low signal intensity on T1-weighted and iso-intensity on T2-weighted images compared with the muscle signal. Axial gadolinium-enhanced T1-weighted MRI with fat suppression revealed an inhomogeneous enhancement within the tumor (Fig. 1B). The MRI revealed no obvious lesions within the pituitary gland. Distal pancreatectomy was performed after the clinical diagnosis of a neuroendocrine tumor with ectopic secretion of ACTH.

The protocol for this study was approved by the Ethics Committee of Tohoku University School of Medicine, Sendai, Japan. Informed consent was obtained from the patient.

2.2. Immuno-histochemistry

For immuno-histochemical studies, paraffin-embedded tissue sections were incubated with antibodies against CD34 (Nichirei Bioscience, Tokyo, Japan, 1:200), bcl-2 (Dako, Copenhagen, Denmark, 1:50), neuron-specific enolase (NSE) (Dako, 1:200), STAT6 (sc-621; Santa Cruz Biotechnology, Santa Cruz, CA, 1:1000), ACTH (Leica Biosystems, Newcastle, UK, 1:800), POMC (Abcam, Cambridge, UK, 1:200), chromogranin A (Dako, 1:100), and synaptophysin (Dako, 1:300). Antigen retrieval was performed using an autoclave (120°C, 5 min) in citrate buffer (10 mmol, pH 6.0) for NSE, STAT6, POMC, and synaptophysin, and using a microwave in citrate buffer (10 mmol, pH 6.0) for bcl-2 and chromogranin A. We did not perform any antigen retrieval for CD34 and ACTH. Immunostaining was performed employing the streptavidin-biotin amplification method. Reacted sections were visualized using 3’,3’- diaminobenzidine-tetrachloride and 30% H2O2 in Tris buffer (0.05 mol, pH 7.6) and were counterstained with hematoxylin.

![Fig. 1. CT, MRI, and gross, histochemical, and immuno-histochemical findings. A: Contrast-enhanced computed tomography shows a well-circumscribed tumor 6 cm in diameter with an inhomogeneous enhancement near the pancreatic tail. There was no defined adrenal tumor. B: Axial gadolinium-enhanced T1-weighted magnetic resonance image with fat suppression shows an inhomogeneous enhancement in the tumor. C: Cut surface of the surgical specimen shows firm mass attached to the pancreas. D: Spindle neoplastic cells in fascicular arrangement (HE, hematoxylin and eosin; ×200). E, F: Positive immunostaining for CD34 and STAT6 (×200). G–I: Focal positive staining for adrenocorticotropic hormone (ACTH; ×200), proopiomelanocortin (POMC; ×200), and neuron-specific enolase (NSE; ×200).](image-url)
2.3. Quantitative real-time polymerase chain reaction

Specimens were snap-frozen for RNA isolation and stored at −80 °C until use. Total RNA was carefully extracted using the RNeasy Mini Kit (QIAGEN, Hilden, Germany) after cryostat disruption at −20 °C. Complementary DNA (cDNA) was produced with QuantiTect reverse transcription kit (QIAGEN) and quantitative real-time polymerase chain reaction (qRT-PCR) was performed with LightCycler FastStart DNA Master SYBR Green I kit (Roche, Basel, Switzerland) in a LightCycler (Roche). The forward and reverse primer sequences for POMC are 5’-CAGCCAGTGTCAGGACCTC-3’ and 5’-GGTCAGAGGCTGCTCGTC-3’ (Rod et al., 2009). qRT-PCRs for identification of recurrent NAB2-STAT6 gene fusions were performed in 3 × 3 ways of primer combination (Chmielecki et al., 2013; Robinson et al., 2013). Forward primers for exons 3, 6, and 7 of NAB2 are NAB2-exon 3, 5’-CCGAGAGGCACTACTTGTCC-3’; NAB2-exon 6, 5’-GACGGGGGGCTCGGCTCGCCC-3’; NAB2-exon 7, 5’-AGTTCGAGGAAGGGCTGCTGG-3’. Reverse primers for exons 3, 17, and 18 of STAT6 are STAT6-exon 3, 5’-CGCTGCACTTTTTCTGGGGG-3’; STAT6-exon 17, 5’-GAGGGGGTTTTAGTGTTGGGGTTATTTGT-3’; STAT6-exon 18, 5’-TCTTCTTGGTTATGCTGGG-3’. The melting curve analyses were performed to verify the amplification of the expected sequence. Negative control experiments did not contain cDNA substrate to check for the possibility of exogenous contaminant DNA, and no amplification was detected under these conditions. In qRT-PCR for POMC and ACTH, the relative mRNA expression was calculated as the ratio of the quantity of POMC and ACTH to house-keeping gene RPL13A cDNA transcripts, as previously described (Felizola et al., 2013). Direct DNA sequencing of amplified products was carried out with ABI BigDye terminator ver.3.1 (Applied Biosystems, Foster City, CA) and ABI Prism 3100-Avant Genetic analyzer (Applied Biosystems).

2.4. DNA methylation analysis of the POMC promoter region

DNA methylation levels at the POMC promoter region were assessed by using PyroMark Q24 (QIAGEN), according to the manufacturer’s protocols. Genomic DNA was extracted from the formalin-fixed and paraffin-embedded section of the tumor or normal pancreas tissue. Bisulfite conversion of genomic DNA and purification of modified DNA was conducted using EZ DNA Methylation-Gold Kit (ZYMO Research, USA), according to manufacturer’s instructions. PCR primers for pyrosequencing were designed using the PyroMark Assay Design (QIAGEN, ver. 2.0.1.15). The PCR and pyrosequencing primers for CpG sites tested within the POMC promoter region are POMC_F1: 5’-GAGGGGTTTTTGGAGGG-3’, POMC_R1: 5’-Biotin)-ACAATACTAATTCACCCCGCTCCG-3’, POMC_Seq1: 5’-GAGGGGGTTTTTGTAG-3’, POMC_Seq2: 5’-GGTITAGGGGAGCTGCCCTGG-3’, and POMC_Domain IV_F1: 5’-TGTGGGGGTGTTATTTGTT-3’, POMC_Domain IV_R1: 5’-(Biotin)-CCITCCCTCCAAAATAA-3’, POMC_Domain IV_Seq1: 5’-GGGTTGTATTGT-3’.

3. Results

The cut surface of the resection specimen revealed a tumor with a diameter of 6 cm, which appeared white—gray and well demarcated (Fig. 1C). On histological examination, the tumor showed spindle cells in a fascicular arrangement. Immunohistochemically, the tumor stained diffusely for STAT6, CD34, and bcl-2; it stained focally for ACTH, POMC, and NSE (Fig. 1D). The tumor was negative for chromogranin A and synaptophysin. The cDNA sequencing of the tumor identified the NAB2-STAT6 fusion gene (Fig. 2A). Using qRT-PCR, we also identified the mRNA that encoded POMC (Fig. 2B). The relative mRNA levels for POMC and ACTH to

**Fig. 2.** cDNA sequencing, qRT-PCR, and DNA methylation analyses. A: Sequencing trace shows the chimeric junction between NAB2 and STAT6. B: Quantitative real-time polymerase chain reaction for proopiomelanocortin (POMC). C: DNA methylation level of the POMC promoter of the normal pancreatic tissue and pancreatic solitary fibrous tumor (SFT). The POMC gene is located on chromosome 2p23.2, spans 8 kb, and contains three introns and four exons, indicated by boxes. Filled circles indicate individual CpG sites, which is assessed in this study. Numbering is relative to the transcription start site (+1) of the POMC gene (RefSeq_NM_001033526.2).
<table>
<thead>
<tr>
<th>Case</th>
<th>Author, Year</th>
<th>Age/ Sex</th>
<th>Symptoms</th>
<th>Size (cm)/Site</th>
<th>Presurgical diagnosis</th>
<th>Surgery</th>
<th>Gross</th>
<th>Immunohistochemistry</th>
<th>Follow-up</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Luttges et al., 1999s</td>
<td>50/Female</td>
<td>Asymptomatic</td>
<td>5.5/Body</td>
<td>NET</td>
<td>DP</td>
<td>Well demarcated</td>
<td>Positive: CD34, CD99, bcl-2, vimentin Negative: SMA, S100</td>
<td>Alive and well after 20 months</td>
</tr>
<tr>
<td>2</td>
<td>Chatti et al., 2006</td>
<td>41/Male</td>
<td>Abdominal pain</td>
<td>13.0/Body</td>
<td>NET</td>
<td>Enucleation</td>
<td>Well demarcated, necrosis</td>
<td>Positive: CD34, CD99, bcl-2, vimentin, SMA (focal), CD117 (focal) Negative: EMA, cytokeratin, S100</td>
<td>Died after 3 days postoperative due to complications</td>
</tr>
<tr>
<td>3</td>
<td>Miyamoto et al., 2007</td>
<td>41/Female</td>
<td>Abdominal pain</td>
<td>2.0/Head-body</td>
<td>NET</td>
<td>Laparoscopic enucleation</td>
<td>Well demarcated, thin capsule</td>
<td>Positive: CD34, bcl-2 Negative: SMA, desmin, S100, CD117, AE1/3, CAM5.2</td>
<td>Alive and well after 7 months</td>
</tr>
<tr>
<td>4</td>
<td>Gardini et al., 2007</td>
<td>62/Female</td>
<td>Abdominal pain</td>
<td>3.0/Head</td>
<td>N.A</td>
<td>PPPD</td>
<td>Well demarcated</td>
<td>Positive: CD34, CD99, bcl-2, vimentin, SMA (focal) Negative: desmin, CD117, S100</td>
<td>Alive and well after 16 months</td>
</tr>
<tr>
<td>5</td>
<td>Srinivasan et al., 2008</td>
<td>78/Female</td>
<td>Back pain, weight loss</td>
<td>5.0/Body</td>
<td>NET</td>
<td>DP</td>
<td>Well demarcated</td>
<td>Positive: CD34(focal), CD99, bcl-2 Negative: SMA, desmin, TdT, CD117, S100, CD10</td>
<td>Alive and well after 7 months</td>
</tr>
<tr>
<td>6</td>
<td>Kwon et al., 2008</td>
<td>54/Male</td>
<td>Asymptomatic</td>
<td>7.6/Body</td>
<td>NET or SPN</td>
<td>Median segmentectomy</td>
<td>Encapsulated, hemorrhage, cystic change</td>
<td>Positive: CD34, CD99 Negative: CD117, S100</td>
<td>N.A</td>
</tr>
<tr>
<td>7</td>
<td>Ishiwatari et al., 2009</td>
<td>58/Female</td>
<td>Asymptomatic</td>
<td>3.0/Head</td>
<td>NET</td>
<td>PPPD</td>
<td>Well demarcated, capsule (partial)</td>
<td>Positive: CD34, bcl-2 Negative: CD99, CD117, vimentin, AE1/3, SMA, S100, desmin, synaptophysin</td>
<td>Alive and well after 42 months</td>
</tr>
<tr>
<td>8</td>
<td>Chetty et al., 2009</td>
<td>67/Female</td>
<td>Asymptomatic</td>
<td>2.6/Head</td>
<td>NET</td>
<td>PD</td>
<td>Well demarcated</td>
<td>Positive: CD34, CD99, bcl-2 Negative: AE1/3, CAM5.2, S100, CD117, SMA, desmin, synaptophysin, chromogranin, β-catenin</td>
<td>Alive and well after 6 months</td>
</tr>
<tr>
<td>9</td>
<td>Sugawara et al., 2010</td>
<td>55/Female</td>
<td>Asymptomatic</td>
<td>7.0/Head</td>
<td>N.A</td>
<td>SSPD</td>
<td>Well demarcated, noncapsulated</td>
<td>Positive: CD34 Negative: SMA, S100, CD117, ALK, cytokeratin</td>
<td>N.A</td>
</tr>
<tr>
<td>10</td>
<td>Tasdemir et al., 2012</td>
<td>24/Female</td>
<td>Abdominal pain</td>
<td>18.5/Head</td>
<td>Mesenchymal tumor</td>
<td>Enucleation</td>
<td>Well demarcated, capsule</td>
<td>Positive: CD34, vimentin, SMA (focal), bcl-2 (focal), β-catenin Negative: S100, CD117, desmin, cytokeratin</td>
<td>Alive and well after 3 months</td>
</tr>
<tr>
<td>11</td>
<td>Vorst et al., 2012</td>
<td>67/Female 49/Female</td>
<td>Abdominal pain</td>
<td>2.8/Head</td>
<td>NET</td>
<td>Enucleation</td>
<td>Well demarcated</td>
<td>Positive: CD34, CD99, bcl-2 Negative: CD117, β-catenin</td>
<td>N.A</td>
</tr>
<tr>
<td>12</td>
<td>Chen et al., 2013</td>
<td>67/Female 49/Female</td>
<td>Abdominal pain</td>
<td>13.0/Head</td>
<td>NET</td>
<td>PD</td>
<td>Well demarcated</td>
<td>Positive: vimentin, CD34, bcl-2, CD68 Negative: cytokeratin, SMA, desmin, CD117, CD99, S100</td>
<td>Alive and well after 30 months</td>
</tr>
<tr>
<td>13</td>
<td>Murakami 2016</td>
<td>82/Male</td>
<td>Hypokalemia, hypertention, edema</td>
<td>6.0/Tail</td>
<td>NET</td>
<td>DP</td>
<td>Well demarcated</td>
<td>Positive: CD34, bcl-2, STAT6, ACTH (focal), POMC (focal), NSE (focal) Negative: chromogranin A, synaptophysin</td>
<td>Died after 4 months postoperative due to sepsis</td>
</tr>
</tbody>
</table>

ACTH, adrenocorticotropic hormone; DP, distal pancreatectomy; EMA, epithelial membrane antigen; N.A, not available; NET, neuroendocrine tumor; NSE, neuron-specific enolase; PD, pancreaticoduodenectomy; POMC, proopiomelanocortin; PPPD, pylorus preserving pancreaticoduodenectomy; SMA, smooth muscle actin; SPN, solid pseudopapillary neoplasm; SSPD, subtotal stomach-preserving pancreaticoduodenectomy.
Defective expression of the transcriptional repressor WT1 (Hajdu)

mechanisms of IGF-II up-regulation is through loss of imprinting, imprinted with the expression of the sole paternal allele. The expression and secretion of IGF-II. The IGF-II gene is normally the development of the hypoglycemia has been linked to the stromal tumors, various types of sarcomas, and SFTs. One reason of mor types, such as hepatocellular carcinoma, gastrointestinal hypoglycemia and often occurs secondary to a wide variety of tu-

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POMC produced in a tumor must be cleaved to release bioactive level of the POMC

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those

secretory pathway (Lacaze Masmonteil et al., 1987). In EAS, of gene encoding the region for the signal peptide required to enter produced by non-neoplastic tissues other than pituitary gland neoplastic pancreatic tissue (Fig. 2C). The post-operative serum Domain IV were hypomethylated in the SFT compared to non-neoplastic tissue, including a NeuroD1 binding site, which is designated as

RPL13A were 2.45 × 10⁻³ and 1.49 × 10⁻³ in pancreatic SFT, respectively. Four CpG sites in the POMC promoter upstream re-

region, including a NeuroD1 binding site, which is designated as

Domain IV of the POMC promoter region is critical for the POMC gene activation (Newell Price et al., 2001). In the pancreatic SFT of this patient, the Domain IV was specifically hypomethylated as compared to non-neoplastic pancreatic tissue. In summary, pancreatic SFT represents a new cause of EAS. SFTs might have features of partial endocrine differentiation.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

Conflicts of interest and source of funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Acknowledgements

We thank Ms. Kumi Kikuchi and Ms. Kazue Ise in the Tohoku University, Sendai, Japan for their excellent technical assistance.

References


Lacaze-Masmonteil, T., de Keyzer, Y., Lutton, J.P., Kahn, A., Bertagna, X., 1987. Characterization of POMC, is found in the prenatal period (von Dorsche et al., 1992). The widespread expression of POMC peptides in such a large number of normal tissues suggests that POMC production by a tumor may represent tumor-induced amplification of POMC in cells from which the tumor originated. However, these POMC peptides produced by non-neoplastic tissues other than pituitary gland cannot be seeretted into the extracellular space because of the lack of gene encoding the region for the signal peptide required to enter the secretory pathway (Lacaze Masmonteil et al., 1987). In EAS, POMC produced in a tumor must be cleared to release bioactive ACTH. However, it awaits further investigation for clarification with regard to the mechanism.

Non-islet-cell tumor hypoglycemia is a rare cause of recurrent hypoglycemia and often occurs secondary to a wide variety of tu-

mor types, such as hepatocellular carcinoma, gastrointestinal stromal tumors, various types of sarcomas, and SFTs. One reason of the development of the hypoglycemia has been linked to the expression and secretion of IGF-II. The IGF-II gene is normally implanted with the expression of the sole paternal allele. The mechanisms of IGF-II up-regulation is through loss of imprinting, inappropriate activation of the morphogen sonic hedgehog, or defective expression of the transcriptional repressor WTI (Hajdu et al., 2010).

DNA methylation is generally associated with transcriptional silencing or decreased gene expression. Methylation level of the POMC promoter region precisely correlates with the expression level of the POMC gene in non-neoplastic pancreatic tissue. The Domain IV of the POMC promoter region is critical for the POMC gene activation (Newell Price et al., 2001). In the pancreatic SFT of this patient, the Domain IV was specifically hypomethylated as compared to non-neoplastic pancreatic tissue. In summary, pancreatic SFT represents a new cause of EAS. SFTs might have features of partial endocrine differentiation.


