Two Case Reports of Pilot Percutaneous Cryosurgery in Familial Multiple Endocrine Neoplasia Type 1

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Abstract: We report 2 cases of familial multiple endocrine neoplasia type 1 syndrome (MEN 1) in related Malaysian Chinese individuals: the son had simultaneous primary lesions in the pancreatic tail, parathyroid, adrenal gland, and hypophysis, with metastatic tumors in the left lung, mediastinum and spine; his mother had simultaneous primary lesions in the pancreatic head, parathyroid, and hypophysis, with metastatic tumors in the liver, spine, ilium, chest wall, and rib. Genetic testing of the 2 patients showed the same mutation in exon 9 of *MEN1* (c.1288G>T, Glu430, encoding a stop codon). The tumors with the poorest prognosis and clinical sequelae were in the pancreas of both patients, and these were treated by percutaneous cryoablation. The number of hypoglycemic episodes in the son improved for more than 120 days, and the abdominal space occupying lesion resolved in his mother.

Key Words: multiple endocrine neoplasia, familial tumor, percutaneous cryosurgery

Abbreviation: MEN 1 - multiple endocrine neoplasia type 1 syndrome, USB - B ultrasound, CT - computed tomography, TAE - transarterial embolization, panNET - pancreatic neuroendocrine tumor, PDAC - pancreatic ductal adenocarcinoma

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Since the first case report of multiple endocrine tumors in 1903 by Erdheim, the definition of this disease has been debated. In 1968, Steiner introduced the term multiple endocrine neoplasia (MEN) to describe disorders that featured combinations of endocrine tumors. This disease was then divided into Wermer syndrome (MEN 1) and Sipple syndrome (MEN 2). Multiple endocrine neoplasia type 1 syndrome is now known to be an autosomal inherited disease, which is diagnosed on the basis of tumors located in at least 2 of the 3 main MEN 1–related endocrine glands: the parathyroids, enteropancreatic tissue, and pituitary gland. In 2001, Crabtree et al[1] confirmed a direct correlation between the *MEN1* gene and MEN 1 with the use of a mouse knockout model. Carney proposed the diagnostic criteria for familial MEN 1 in 2005,[2] played an instrumental role in discerning the screening of this disease[3,4] and the selection of appropriate therapies. At present, some patients who have minimal symptoms can be cured by surgery,[5–7] arterial chemoembolization[8] or the combined application of both methods,[9] or combined treatments with hormones and surgery.[10] The quality of life of affected patients can be alleviated significantly by these methods, but those patients with MEN 1 who have widespread metastases cannot undergo surgery, chemotherapy, and radiotherapy and currently lack a criterion standard therapy. In this study, percutaneous cryosurgeries with an argon-helium cryogenic system under the guidance of B ultrasound (USB) or computed tomography (CT) imaging were applied to alleviate 2 members of a family with MEN 1 who had widespread metastases.

MATERIALS AND METHODS

Case 1

A 23-year-old man of Malaysian Chinese origin was referred to our hospital on June 9, 2011 with a history of obviously decreased physical strength and multiple hypoglycemic comas. The patient was obese (body mass index [BMI], 31.14 kg/m^2), with a poor mental state and occasionally dyspneic. Fasting blood glucose varied from 1.2 to 2.2 mmol/L (reference range, 3.9–6.1 mmol/L); serum fasting C peptide was 21.8 ng/mL (reference range, 1.1–4.4 ng/mL), insulin was 109.5 μU/mL (reference range, 2.6–24.9 μU/mL), pituitary prolactin was 34.7 ng/mL (reference range, 2.1–17.7 ng/mL), thyroid-stimulating hormone was 30.7 μIU/L (reference range, 0.3–4.2 μIU/L), and his parathyroid hormone level was 12.4 pmol/L (reference range, 1.30–6.80 pmol/L), which were all significantly higher than their normal ranges. The levels of gastrin, calcitonin, CA19-9, and adrenal gland and thyroid-associated hormones were all in the normal ranges; serum ionized calcium was increased (1.43 mmol/L; reference range, 1.1–1.34 mmol/L), whereas phosphate levels were decreased (0.47 mmol/L; reference range, 0.81–1.62 mmol/L).

Chest and abdominal CT scans revealed a pancreatic tail tumor (3.4 × 3.4 cm; Fig. 1A), a huge left frontal mediastinal lymph node metastasis (5.4 × 4.7 cm), and metastases to the left adrenal gland (1 × 1 cm), lung, pelvis and spine, without visible tumors in the pituitary hypophysis or thyroid. Pancreatic tumor biopsies were performed under the guidance of CT scans, and immunochemical analysis demonstrated that the tumors were positive for synaptophysin, chromgranin A, carcinoembryonic antigen, and Ki67 but negative for cytokaratin, vimentin, and S100 protein (Fig. 1B). This led to the diagnosis of a pancreatic neuroendocrine tumor (panNET).

As a result of the pancreatic tail panNET, high levels of insulin and C peptide and low blood glucose levels, the patient's condition was diagnosed as insulinoma. To confirm whether the

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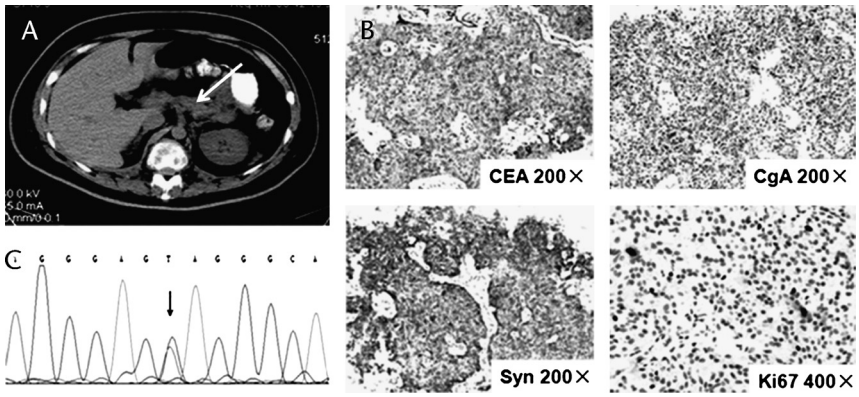

FIGURE 1. Main differential diagnosis of the son. A, Computed tomographic scanning of the abdomen; the red arrow shows the tumor in the pancreatic tail. B, panNET-associated immunochemical analysis for the pancreatic tail tumor; the brown-dyed cells are positive for different indexes, and all nuclei are dyed by hematoxylin. C, Mutation analysis of the *MEN1* gene; the black arrow shows the twin peaks showing the mutation at exon 9.

patient had MEN 1, a peripheral blood sample was collected for the genotyping of *MEN1*. Ten exons of the *MEN1* gene were amplified, and a c.1288C>T heterozygous mutation (Glu430; Fig. 1C) was identified in exon 9. This mutation resulted in the change from glutamic acid to a stop codon and the termination of *MEN1* translation. Therefore, the patient's condition was diagnosed as MEN 1.

As the patient had widespread metastases with intermittent coma and breathing difficulties, he was not a suitable candidate for treatment by surgery, chemotherapy, or radiotherapy; percutaneous cryosurgery was therefore recommended. Owing to the mediastinal lymph node metastasis that caused dyspnea, it was decided that this should be treated initially; the highly functional insulinoma in the pancreatic tail was initially managed by a continuous glucose transfusion, and the adrenal tumor that was compressing the left kidney was treated by a second course of cryoablation, along with the pancreatic tail tumor.

Cryosurgery of the mediastinal lymph nodes was performed on June 16. Under the guidance of USB, 4 cryoprobes (1.7- and 2.0-mm diameter, two of each) were inserted into the mediastinal lymph node, and 100% argon was applied for 10 minutes. The target zone was observed to be covered by an ice ball under USB. The probes were then naturally rewarmed to room temperature, and 2 cycles were conducted in total. The probes were retracted after they were heated by helium, and the puncture points were packed. This procedure was similar for each time of cryosurgery. The patient was able to breathe easily after cryoablation. A CT scan in July 12 showed that the mediastinal lymph node had swollen (7.1 × 4.8 cm), but a later scan on September 28 showed that the gland was smaller (6.3 × 3.6 cm) with reduced density that was indicative of necrosis.

Cryosurgeries of the pancreatic tail and the left adrenal gland were performed on July 17. Under CT guidance, three 1.7-mm diameter cryoprobes were inserted into the pancreatic tail tumor (Fig. 2A), and 100% argon was applied for 10 minutes. The target zone was covered by an ice ball and the cycle was repeated. For the adrenal gland tumor, a 1.7-mm diameter probe was placed into the tumor, 100% argon was applied for 5 minutes, and most of the target zone was covered by an ice ball.

After cryosurgery, antigrowth resistance therapy was applied (Sandostatin, 100 mg per 8 hours, hypodermic injection). The glucose transfusion was reduced gradually after cryosurgery and stopped on day 10 postoperatively (Fig. 2B). The patient's fasting blood glucose dropped to 2 mmol/L 4 days postoperatively, rose to 12 mmol/L in the subsequent 8 days, and then returned to within the normal limits (Fig. 2C); the fasting insulin and C peptide also dropped gradually and returned to normal limits within 12 or 14 days after pancreatic tail cryosurgery (Fig. 2D). A CT scan on August 12 showed that the pancreatic tail tumor had been replaced by an area of low-density necrosis, and the left adrenal gland was swollen (3.6 × 3 cm) and contained low-density necrosis (Fig. 2E).

The patient's physical strength and energy improved significantly, and he was soon able to walk and run freely. He was discharged on Aug 28. When he returned for a follow-up physical examination on September 28, he had even better physical strength and energy, and his body weight had reduced by 10 kg (BMI, 27.68 kg/m^2). A CT scan of the abdomen showed low density in the pancreatic tail (Fig. 2F). Blood glucose monitoring ceased 120 days postoperatively, when the levels were continuously normal.

Case 2

The mother of the aforementioned case patient, a 59-year-old woman of Malaysian Chinese origin was referred to our hospital complaining of gradually decreased body weight and obvious hard masses on her right upper abdomen and left axilla. She was hospitalized as a result of her poor physical state and malaise. Her BMI was 17.52 kg/m^2. Laboratory analyses revealed her serum ionized calcium was 1.45 mmol/L, and her phosphate was 0.79 mmol/L, which were both lower than the reference range; the level of calcitonin was 15.33 nmol/L (reference range, 0–1.46 nmol/L), parathyroid hormone was 36.4 nmol/L, and pituitary prolactin was 40.9 ng/dL, which were all higher than normal limits. Serum fasting C peptide, insulin, gastrin, CA19-9, adrenal gland and thyroid-associated hormones were all in the reference ranges. Ultrasound B, CT, and magnetic resonance imaging all demonstrated a huge tumor at the pancreatic head (10 × 9 cm; Fig. 3A), and multiple tumors in the spine, pelvis, left side of the chest wall (4 × 3 cm; Fig. 3B), liver and ribs, without visible tumors in the hypophysis or thyroid gland on CT imaging. Biopsies of tumors on the left side of the chest wall tumor biopsies were performed under CT guidance. Immunochemical analyses of these were positive for synaptophysin, chromgranin A, cytokaratin, vimentin, and CD56, whereas they were negative for D2-40 and S100 protein (Fig. 3C), which helped to diagnose the panNET. Gene mutation testing also showed the same heterozygous mutation in exon 9 as her son (Fig. 3D).

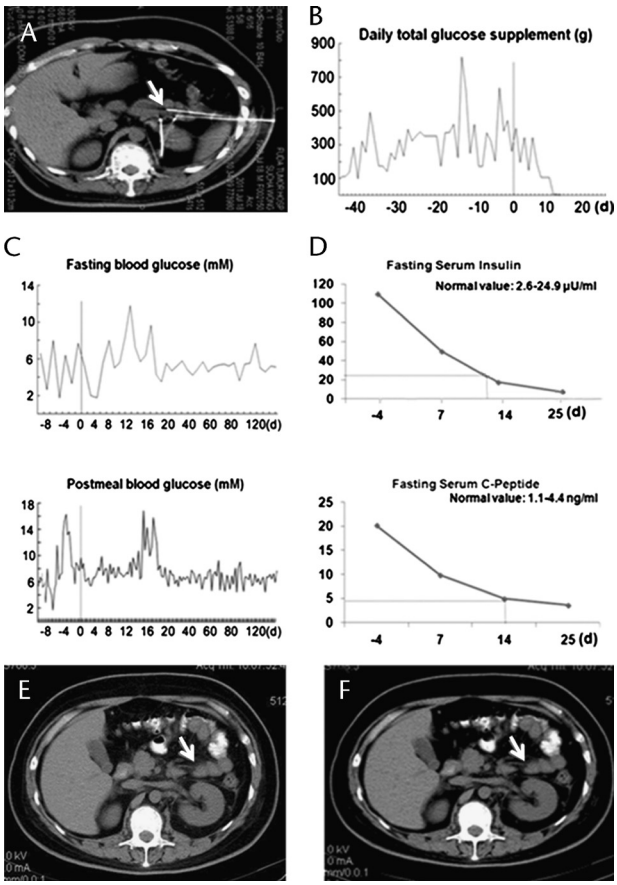

FIGURE 2. Main treatment process and curative effects in the son. A, Cryoablation of the tumor under CT guidance; the arrow shows the tumor at the pancreatic tail, and 3 white lines represent the cryoprobes. B, Daily total supplementation of glucose before and after cryosurgery; the vertical line at day 0 is the day of cryosurgery. C, Serum fasting and postmeal blood glucose levels before and after cryosurgery; the vertical line at day 0 is the day of cryosurgery. D, Serum fasting insulin and C peptide levels before and after cryosurgery; the red lines shows the first day after cryosurgery after which the index returned to normal level. E, Computed tomographic scanning of the abdomen 25 days after cryosurgery; the arrow shows the primary tumor site. F, CT scanning of the abdomen 2 months after cryosurgery; the arrow shows the primary tumor site.

According to the huge panNET on the pancreatic head, the pathological changes in the pituitary and parathyroid gland, and the mutation in *MEN1*, this patient's condition was diagnosed as MEN 1, and the family was confirmed to have the familial MEN 1 syndrome. Owing to the normal levels of serum CA19-9, fasting insulin, and C peptide, the panNET on the pancreatic head was diagnosed as a nonfunctioning MEN 1 tumor. As a result of the severe wasting and physical weakness, the patient was not deemed to be suitable for a thoracotomy, laparoscopic surgery, chemotherapy, and radiotherapy; percutaneous cryosurgery was therefore recommended. The tumors on the left side of the chest wall and pancreatic head were significantly larger than the other metastases and caused severe pressure symptoms to the lung and abdomen, respectively. Therefore, these underwent the first cryoablation procedure. As a result of the excessive volume of tumor on the pancreatic head, this was treated by transarterial embolization (TAE) first and then by cryosurgery.

Cryosurgery of the left side of the chest wall was performed on July 7. Under CT and USB guidance, two 1.7-mm-diameter cryoprobes and six 2-mm-diameter cryoprobes were inserted into the tumor of the left side of the chest wall, with 3 freezing cycles of 10 minutes. On July 27 and August 9, 2 TAE procedures were carried out on the pancreatic head tumor; the pancreaticoduodenal artery and gastroduodenal branch arteries were targeted, respectively. A CT scan on August 22 showed an 8.0 × 8.9-cm soft tissue mass on the pancreatic head and large embolized peripheral image, which resolved well. As the physical condition was well, the patient was discharged from hospital on August 28 to await cryosurgery.

When she was readmitted on September 25, she had gained physical strength and energy and her body weight had increased by 1 kg already. A CT scan of her abdomen showed good sedimentation of the iodipin (Fig. 4A). Cryosurgery of the pancreatic head was performed on November 11. Under USB guidance, four 2-mm-diameter cryoprobes were placed into the pancreatic head tumor, frozen for 15 minutes and then rewarmed; all probes were extracted by 3 cm and frozen for a further 15 minutes. After cryosurgery, antigrowth resistance therapy was applied (Sandostatin, 100 mg per 8 hours, hypodermic injection). An abdominal CT scan on November 29 showed a 10 × 9-cm soft tissue mass on the pancreatic head, which contained a large liquefied necrotic focus (Fig. 4B); the left-sided chest wall tumor had shrunk to a 3 × 2-cm soft tissue mass that had uniform

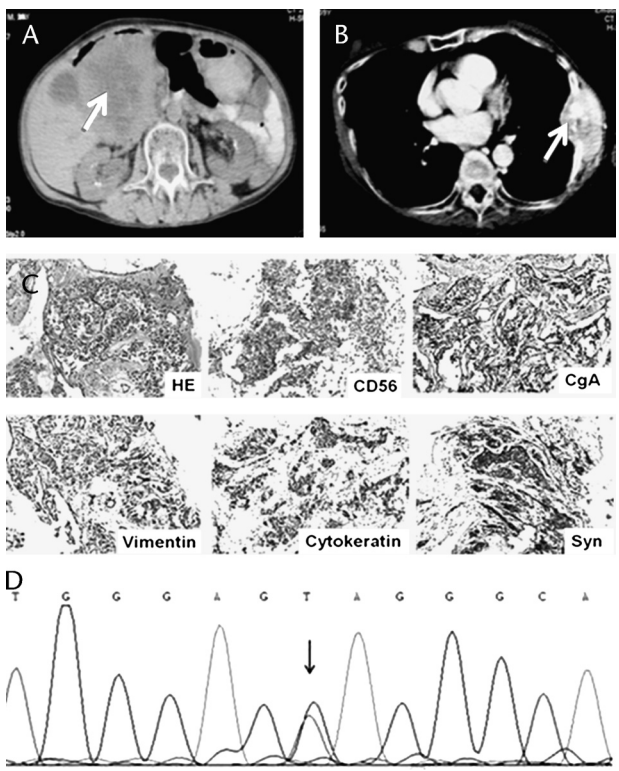

FIGURE 3. Main differential diagnosis of the mother. A, Initial CT scan of the abdomen; the arrow shows the tumor of pancreatic head. B, Initial CT scan of the chest, the arrow shows the tumor of the left chest wall. C, PanNET-associated immunochemistry analysis for the tumor of the left side of the chest wall; the brown-dyed cells are positive for different indexes and all nuclei are dyed by hematoxylin. D, Mutation analysis of the *MEN1* gene; the black arrow shows the twin peaks of the mutation at exon 9.

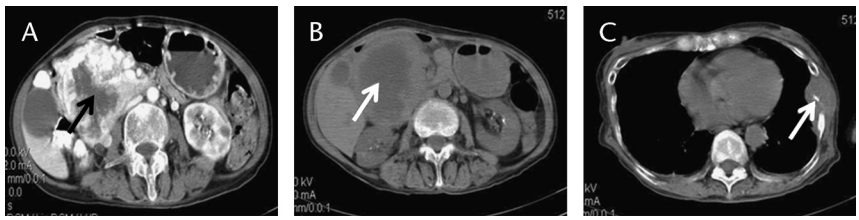

FIGURE 4. Main treatment process and curative effect of the mother. A, CT scanning of the abdomen 2 months after TAE; the arrow shows the tumor of the pancreatic head. B, Computed tomographic scanning of the abdomen 18 days after cryosurgery; the arrow shows the tumor of the pancreatic head. C, Computed tomographic scan of the chest 4.5 months after cryosurgery; the arrow shows the site of the tumor of the left side of the chest wall.

density (Fig. 4C). The patient's physical strength and energy continued to improve, and her body weight increased by 3 kg (BMI, 19.23 kg/m^2) at her latest follow-up.

DISCUSSION

The MEN 1 syndrome usually comprises at least 2 tumors, including parathyroid adenoma, gastrointestinal pancreatic cancer, and pituitary and adrenal tumors; parathyroid adenoma is the most common,[11] followed by pancreaticogastrointestinal pancreatic cancer, which are mainly insulinomas and gastrinomas. Other tumors include glucagonomas and nonfunctioning endocrine tumors, among others.[12–14] Because *MEN1* gene mutations became one of the specific diagnostic criteria for MEN 1, 1336 kinds of point mutation have been found. The most common 9 mutations account for only 20% of all embryonic cell mutations,[15] so sequencing the gene is useful to confirm the specific mutation. The c.1288C>T mutation that was found in the 2 members of this family represented a novel point mutation and strongly suggested the diagnosis of familial MEN 1; the other son has a 50% probability of developing MEN 1. As both of the mother's parents had died, it is difficult to assess whether the c.1288C>T mutation was spontaneous or genetic. However, as the mother had 4 siblings, genetic screening will be of great significance to this large family.

Both members of this family were hospitalized as a consequence of their pancreatic tumor, which were initially diagnosed as pancreatic ductal adenocarcinomas (PDAC); MEN 1 was only diagnosed once genetic mutation tests were performed. Multiple endocrine neoplasia type 1 syndrome is a subtype of the panNET tumor group, which is associated with 44% probability of a *MEN1* gene mutation.[16] The 10-year survival rate of panNET reaches more than 40%,[17,18] whereas the 5-year survival rate for PDAC is less than 2%.[19] It is hard to differentiate between the 2 tumor types on imaging and pathology, and most of the patients with PDAC who survive for more than 10 years after diagnosis are likely to have panNET, and even MEN 1, instead.

As a minimal invasive technology, percutaneous cryosurgery has many advantages compared with conventional surgery. First, percutaneous cryosurgery can locate the tumors precisely, reduce the level of trauma, and has wide indications. It has been applied in the ablation of tumors of the lung,[20] liver,[21] and kidney,[22] among others. Second, percutaneous cryosurgery can activate the cryoimmunity of patients[23]. Third, the simultaneous ablation of primary and metastatic tumors can be performed.[24,25] In the treatment process of the son, the primary tumor on the pancreatic tail and the metastasis to the left adrenal gland were frozen during the same procedure. Fourth, for large tumors, multiple cryoprobes[26] or chemical reagents (eg, alcohol or hydrochloric acid)[27] can be used to expand the frozen area; for tumors with complex anatomical structures (eg, involving the pancreatic head), iodine 125 seed implantation[28,29] and TAE[30] can be combined to reduce damage to the target organs and surrounding normal tissues. Combined with TAE, the huge tumor on the pancreatic head of the mother described in this report was successful frozen with 4 cryoprobes. Kovach et al,[31] and Korpan[32] have both reported the liquid nitrogen freezing of PDAC under laparotomy; they both established that cryoablation was suitable for most cases of PDAC, with lower levels of trauma and lower complication and mortality rates, postoperatively. In 2008, Xu twice reported the clinical trials of PDAC treatment by percutaneous cryosurgery, which have shown the safety and efficacy of this method.[28,29] These provided us with important experiences in pancreatic cryoablation in the 2 patients.

Commonly used evaluation indexes for the treatment of panNET are changes in blood glucose levels, tumor volume, physical strength and energy levels. The main symptoms of both patients have been significantly improved, including the fasting blood glucose of the son, which was maintained within normal limits for more than 120 days. Consistent with the secondary necrosis theory of cryoablation proprosed by Forest et al[33] in 2005, insulin did not return to physiological secretion levels until the secondary necrosis of remaining insulinoma had occurred 4 days postoperatively. Furthermore, the physical strength and energy of the mother improved continuously after cryoablation of the chest wall and the pancreatic head tumors, whereas her severe wasting and weight loss were reversed. These curative effects have provided the time needed in both patients for the sequential cure of their remaining tumors.

In the 1 to 2 months after cryosurgery, multiple frozen sites in both patients seemed to be larger in volume on CT imaging, which may be related to inflammation and swelling. Obvious foci of necrotic liquefaction can be seen on CT imaging 2 months after cryosurgery. The above phenomena are in accordance with the characteristics of cancer cryoablation and are consistent with the cryoablation phenomena observed after the treatment of other kinds of tumors.[24,30] In the huge tumor in the pancreatic head of the mother, a large focus of necrotic liquefaction was achieved within 20 days after the combination treatment of TAE and cryosurgery, which showed that TAE can obviously accelerate the frozen necrosis of tumors.

This is the first report to describe the use of percutaneous cryosurgery for pancreatic and metastatic tumors in patients with familial MEN 1. The preliminary results of blood glucose levels and CT scans have demonstrated the potential curative effects of cryosurgery in the short-term observation period. Percutaneous cryosurgery may offer an alternative treatment method for unresectable pancreatic and metastatic tumors in patients with MEN 1.

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REFERENCES

1. Crabtree JS, Scacheri PC, Ward JM, et al. A mouse model of multiple endocrine neoplasia, type 1, develops multiple endocrine tumors. *Proc Natl Acad Sci U S A*. 2001;98:1118–1123.
2. Carney JA. Familial multiple endocrine neoplasia: the first 100 years. *Am J Surg Pathol*. 2005;29:254–274.
3. Boguszewski CL, Bianchet LC, Raskin S, et al. Application of genetic testing to define the surgical approach in a sporadic case of multiple endocrine neoplasia type 1. *Arq Bras Endocrinol Metabol*. 2010;54:705–710.
4. Skandarajah A, Barlier A, Morlet-Barlat N, et al. Should routine analysis of the MEN1 gene be performed in all patients with primary hyperparathyroidism under 40 years of age? *World J Surg*. 2010;34:1294–1298.
5. Waldmann J, Habbe N, Fendrich V, et al. Fast-growing pancreatic neuroendocrine carcinoma in a patient with multiple endocrine neoplasia type 1: a case report. *J Med Case Rep*. 2008;2:354.
6. Zhou G, Wei Y, Chen X, et al. Multiple endocrine neoplasia type 1 simultaneous with various types of enteropancreatic endocrine tumors: one case report: lessons learned. *Pancreas*. 2010;39:680–683.
7. Lee SR, Choi MC, Ahn KJ. A case of multiple endocrine neoplasia type 1 with primary liver gastrinoma. *J Korean Med Sci*. 2010;25:953–956.
8. Milanesi A, Yu R, Geller SA, et al. Concurrent primary hyperparathyroidism and humoral hypercalcemia of malignancy in a patient with multiple endocrine neoplasia type 1. *Pancreas*. 2011;40:634–637.
9. Tonelli F, Fratini G, Nesi G, et al. Pancreatectomy in multiple endocrine neoplasia type 1-related gastrinomas and pancreatic endocrine neoplasias. *Ann Surg*. 2006;244:61–70.
10. Imamura M, Komoto I, Ota S, et al. Biochemically curative surgery for gastrinoma in multiple endocrine neoplasia type 1 patients. *World J Gastroenterol*. 2011;17:1343–1353.
11. Thakker RV. Multiple endocrine neoplasia type 1 (MEN1). *Best Pract Res Clin Endocrinol Metab*. 2010;24:355–370.
12. Namihira Y, Achord JL, Subramony C. Multiple endocrine neoplasia, type 1, with pancreatic cholera. *Am J Gastroenterol*. 1987;82:794–797.
13. Brandi ML, Gagel RF, Angeli A, et al. Guidelines for diagnosis and therapy of MEN type 1 and type 2. *J Clin Endocrinol Metab*. 2001;86:5658–5671.
14. Triponez F, Dosseh D, Goudet P, et al. Epidemiology data on 108 MEN 1 patients from the GTE with isolated nonfunctioning tumors of the pancreas. *Ann Surg*. 2006;243:265–272.
15. Lemos MC, Thakker RV. Multiple endocrine neoplasia type 1 (MEN1): analysis of 1336 mutations reported in the first decade following identification of the gene. *Hum Mutat*. 2008;29:22–32.
16. Jiao Y, Shi C, Edil BH, et al. DAXX/ATRX, MEN1, and mTOR pathway genes are frequently altered in pancreatic neuroendocrine tumors. *Science*. 2011;331:1199–1203.
17. Ekeblad S, Skogseid B, Dunder K, et al. Prognostic factors and survival in 324 patients with pancreatic endocrine tumor treated at a single institution. *Clin Cancer Res*. 2008;14:7798–7803.
18. Fredrich M, Reisch A, Illing RB. Neuronal subtype identity in the rat auditory brainstem as defined by molecular profile and axonal projection. *Exp Brain Res*. 2009;195:241–260.
19. Siegel R, Ward E, Brawley O, et al. Cancer statistics, 2011: the impact of eliminating socioeconomic and racial disparities on premature cancer deaths. *CA Cancer J Clin*. 2011;61:212–236.
20. Sharma A, Moore WH, Lanuti M, et al. How I do it: radiofrequency ablation and cryoablation of lung tumors. *J Thorac Imaging*. 2011;26:162–174.
21. Chen HW, Lai EC, Zhen ZJ, et al. Ultrasound-guided percutaneous cryotherapy of hepatocellular carcinoma. *Int J Surg*. 2011;9:188–191.
22. Littrup PJ, Ahmed A, Aoun HD, et al. CT-guided percutaneous cryotherapy of renal masses. *J Vasc Interv Radiol*. 2007;18:383–392.
23. Sabel MS. Cryo-immunology: a review of the literature and proposed mechanisms for stimulatory versus suppressive immune responses. *Cryobiology*. 2009;58:1–11.
24. Xu KC, Niu LZ, He WB, et al. Percutaneous cryosurgery for the treatment of hepatic colorectal metastases. *World J Gastroenterol*. 2008;14:1430–1436.
25. Yamauchi Y, Izumi Y, Kawamura M, et al. Percutaneous cryoablation of pulmonary metastases from colorectal cancer. *PLoS One*. 2011;6:e27086.
26. Littrup PJ, Jallad B, Vorugu V, et al. Lethal isotherms of cryoablation in a phantom study: effects of heat load, probe size, and number. *J Vasc Interv Radiol*. 2009;20:1343–1351.
27. Xu KC, Niu LZ, He WB, et al. Percutaneous cryoablation in combination with ethanol injection for unresectable hepatocellular carcinoma. *World J Gastroenterol*. 2003;9:2686–2689.
28. Xu KC, Niu LZ, Hu YZ, et al. A pilot study on combination of cryosurgery and (125)iodine seed implantation for treatment of locally advanced pancreatic cancer. *World J Gastroenterol*. 2008;14:1603–1611.
29. Xu KC, Niu LZ, Hu YZ, et al. Cryosurgery with combination of (125)iodine seed implantation for the treatment of locally advanced pancreatic cancer. *J Dig Dis*. 2008;9:32–40.
30. Xu KC, Niu LZ, Zhou Q, et al. Sequential use of transarterial chemoembolization and percutaneous cryosurgery for hepatocellular carcinoma. *World J Gastroenterol*. 2009;15:3664–3669.
31. Kovach SJ, Hendrickson RJ, Cappadona CR, et al. Cryoablation of unresectable pancreatic cancer. *Surgery*. 2002;131:463–464.
32. Korpan NN. Cryosurgery: ultrastructural changes in pancreas tissue after low temperature exposure. *Technol Cancer Res Treat*. 2007;6:59–67.
33. Forest V, Peoc'h M, Campos L, et al. Effects of cryotherapy or chemotherapy on apoptosis in a non-small-cell lung cancer xenografted into SCID mice. *Cryobiology*. 2005;50:29–37.