Placental site trophoblastic tumor: p53 gene analysis

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Summary

Background: Placental site trophoblastic tumor (PSTT) is the rarest type of trophoblastic neoplasm. Because of its rarity, the clinical behavior and pathogenesis of PSTT are still unclear.

Case: A 20-year-old woman presented with secondary amenorrhea and irregular vaginal bleeding. Examination of the patient revealed elevated serum hCG and a uterine mass. The specimen obtained by curettage was diagnosed as possible PSTT. The patient was treated with two cycles of EMA/CO, but her uterine mass increased in size. Subsequently, she underwent total abdominal hysterectomy. Microscopic observation revealed a PSTT. To estimate the status of expression of p53 protein and to determine whether p53 gene mutation was present in this PSTT, we carried out immunohistochemical staining for p53 and PCR-SSCP analysis. Immunohistochemical staining for p53 revealed intense nuclear labeling, but no p53 gene mutation was detected in exons 5-8.

Conclusion: Analysis of the p53 gene may aid understanding of the pathogenesis of PSTT.

Key words: Placental site trophoblastic tumor; Multiagent chemotherapy; p53.

Introduction

Placental site trophoblastic tumor (PSTT) is the rarest form of gestational trophoblastic disease. Since its original description by Kurman *et al.* [1], approximately 100 cases of it have been reported in the English literature. Because of its rarity, the clinical behavior and pathogenesis of PSTT are still unclear. This report describes a 20-year-old woman with secondary amenorrhea and irregular vaginal bleeding in whom investigations revealed a PSTT. Using surgically removed tissue, we analyzed the expression and mutation status of p53.

Case report

The patient, a 20-year-old gravida 1 para 0 woman, consulted our hospital for genital bleeding and a uterine mass on August 28, 2001. She had undergone a therapeutic abortion in November 2000. She subsequently complained of secondary amenorrhea and vaginal spotting. On August 27, 2001 she visited a gynecologic department and was suspected of having a molar pregnancy based on sonography findings. The patient had otherwise always been in excellent health.

Pelvic examination revealed an enlarged soft uterus of 10 weeks' size. Laboratory investigations revealed that serum human chorionic gonadotropin (hCG) and urinary hCG were elevated to 1080 mIU/ml (normal, < 0.5 mIU/ml) and 260 mIU/ml (normal, < 0.5 mIU/ml), respectively. The serum human placental lactogen (hPL) level was beneath the limit of detection. The tumor markers AFP, CEA, CA-125, CA 19-9 and CA 72-4 were also within normal ranges. Pelvic sonography revealed a 5.5 × 4.0-cm mass in the posterior uterine wall with multiple cystic lesions of several sizes. The cystic lesions were considered to be dilated vessels since blood flow was evident within them on color flow doppler images (Figure 1). Head, chest and abdominal computed tomography revealed no metastatic lesions.

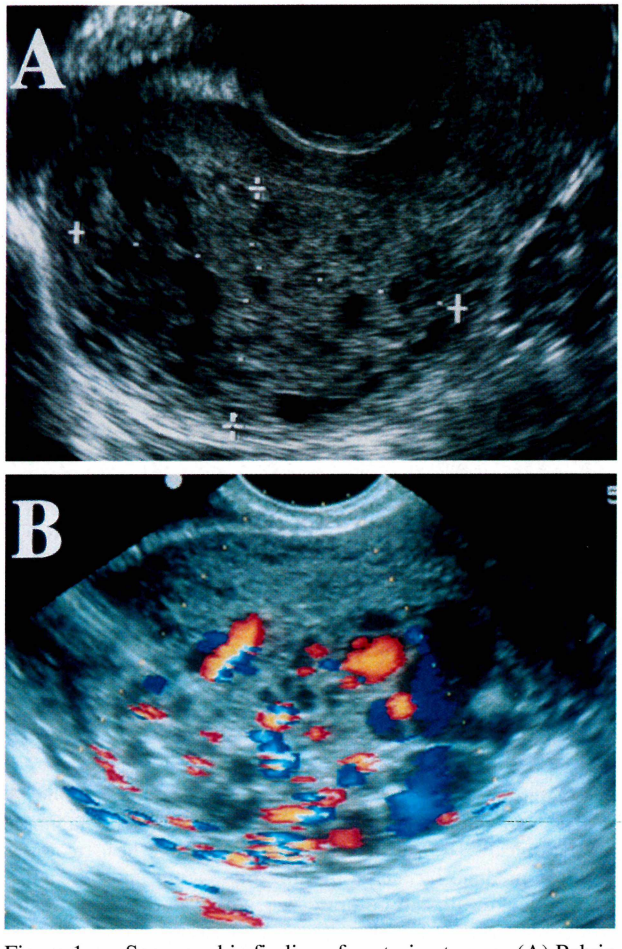

Figure 1. — Sonographic findings for uterine tumor. (A) Pelvic ultrasonography revealed a 5.5 × 4.0 cm mass in the posterior uterine wall with multiple cystic lesions. (B) Color flow doppler image of the tumor.

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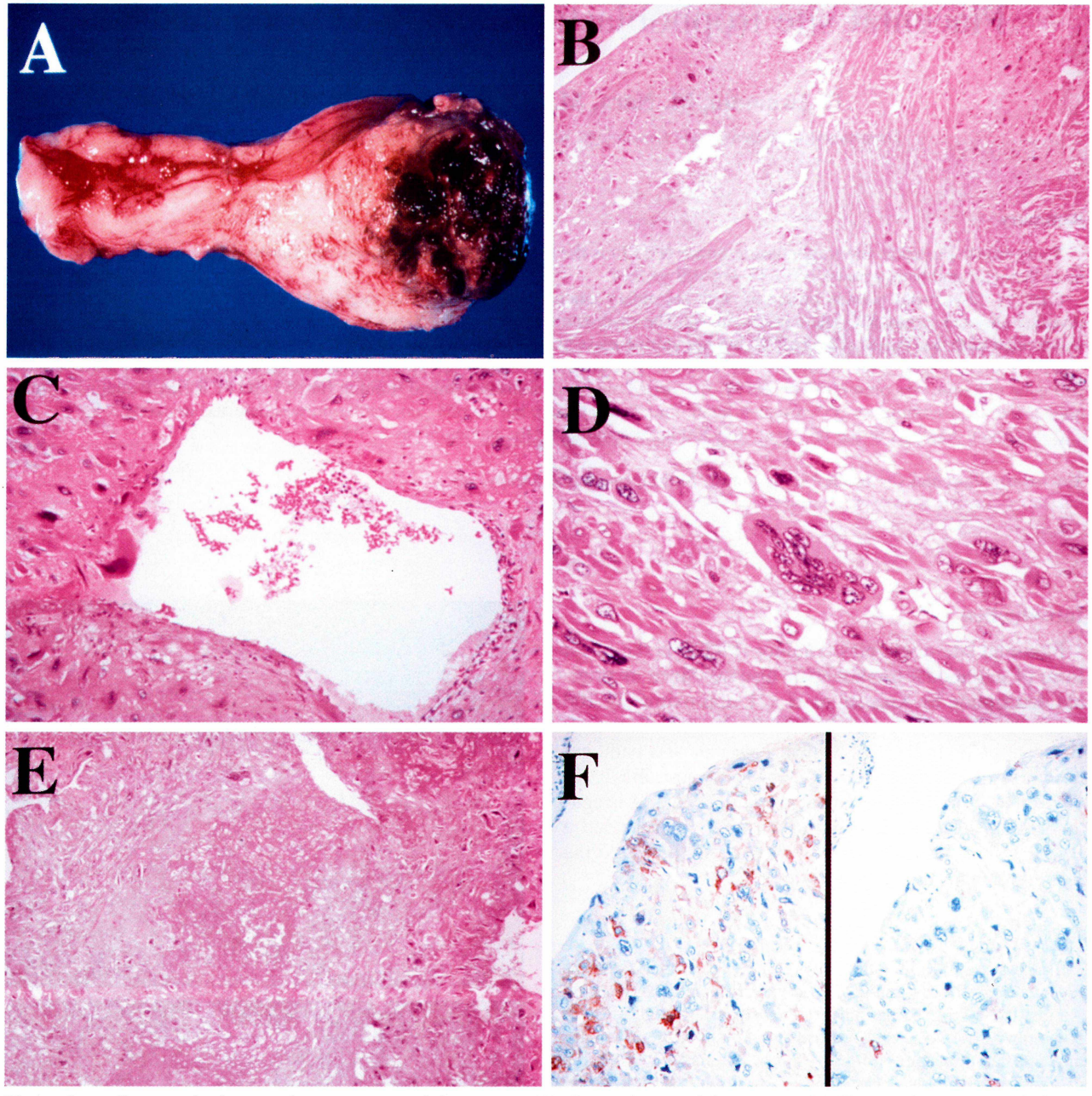

Figure 2. — Gross and microscopic appearance of the tumor. (A) Gross picture of the tumor. (B) Sheets of monomorphic intermediate trophoblasts infiltrated between smooth muscle fibers of the myometrium. (C) The blood vessel wall appeared to have been completely replaced by trophoblastic cells. (D) Multinuclear giant cells were found occasionally. (E) Hemorrhage and necrosis were observed in this case. (F) Immunohistochemical staining was diffusely positive for hPL (left) and focally positive for hCG (right).

Dilatation and curettage were performed on September 4, 2001. A small specimen obtained by curettage was diagnosed as possible PSTT. We therefore considered the best treatment to be immediate hysterectomy, but the patient and her family wished her fertility to be preserved. She was therefore treated with two cycles of EMA/CO chemotherapy (etoposide 100 mg/m^2 IV on days 1 and 2, methotrexate 100 mg/m^2 IV on day 1 followed immediately by a 12-h IV infusion of 200 mg/m^2 methotrexate with leukovorin rescue on days 2 and 3, actinomycin-D 0.5 mg IV on days 1 and 2, and then vincristine 1.0 mg/m^2 IV and cyclophosphamide 600 mg/m^2 IV on day 8). After two cycles of EMA/CO chemotherapy, her serum hCG had decreased to 105 mIU/ml, but the uterine mass had increased in size. At this time, the patient and her family made the decision to undergo hysterectomy. On December 20, 2001 the patient underwent total abdominal hysterectomy. Her serum hCG dropped to within the normal range after hysterectomy. She is alive and well at this time.

Pathological findings for the resected uterus are shown in Figure 2. The cut surface of the resected uterus revealed a

necrotic, soft mass measuring 6 × 5 cm in the posterior uterine wall. The tumor had invaded the entire thickness of the myometrium. Microscopically, the tumor was composed of round or polygonal cells, most of which were mononucleate but some of which were multinucleate. Nuclear atypia was remarkable and nucleoli were small. The monophasic cellular pattern of intermediate trophoblasts, and not the dimorphic structure of cytotrophoblasts and syncytiotrophoblasts, was observed. Villus formation was not observed. The neoplastic cells infiltrated the myometrium in the form of single cell or small cellular aggregates dissecting between muscle fibers. Perivascular areas were often invaded by the neoplastic cells. The mitotic count was about 1~2/10 high-power fields (HPF). Hemorrhage and necrosis, which are usually considered characteristic findings of choriocarcinoma, were observed. On immunohistochemical examination, most of the neoplastic cells were stained with hPL, whereas few were stained with hCG.

To estimate the status of expression of p53 protein in this PSTT, we carried out immunohistochemical staining for p53. Staining was performed as follows: after inhibition of endogenous peroxidase activity, deparaffinized sections were incubated overnight with mouse anti-p53 antibody (DO-1, PharMingen International). Antibody binding was demonstrated by a peroxidase-antiperoxidase technique. Peroxidase was detected by incubation in 3,3'-diaminobenzidine tetrahydrochloride and hydrogen peroxide. As shown in Figure 3, p53 was strongly stained for in the tumor tissue. Furthermore, we carried out polymerase chain reaction-single strand conformation polymorphism (PCR-SSCP) analysis to determine whether p53 gene mutation was present in this case. Template DNA was extracted from paraffin blocks and mutations in exons 5-8 of the p53 gene were screened by PCR-SSCP analysis. As shown in Figure 4, no p53 gene mutation was detected in these exons.

Figure 3. — Immunohistochemical staining for p53.

Discussion

PSTT was originally described in 1976 by Kurman *et al.* in a series of 12 patients, none of whom succumbed to their disease or had evidence of distant spread [1].

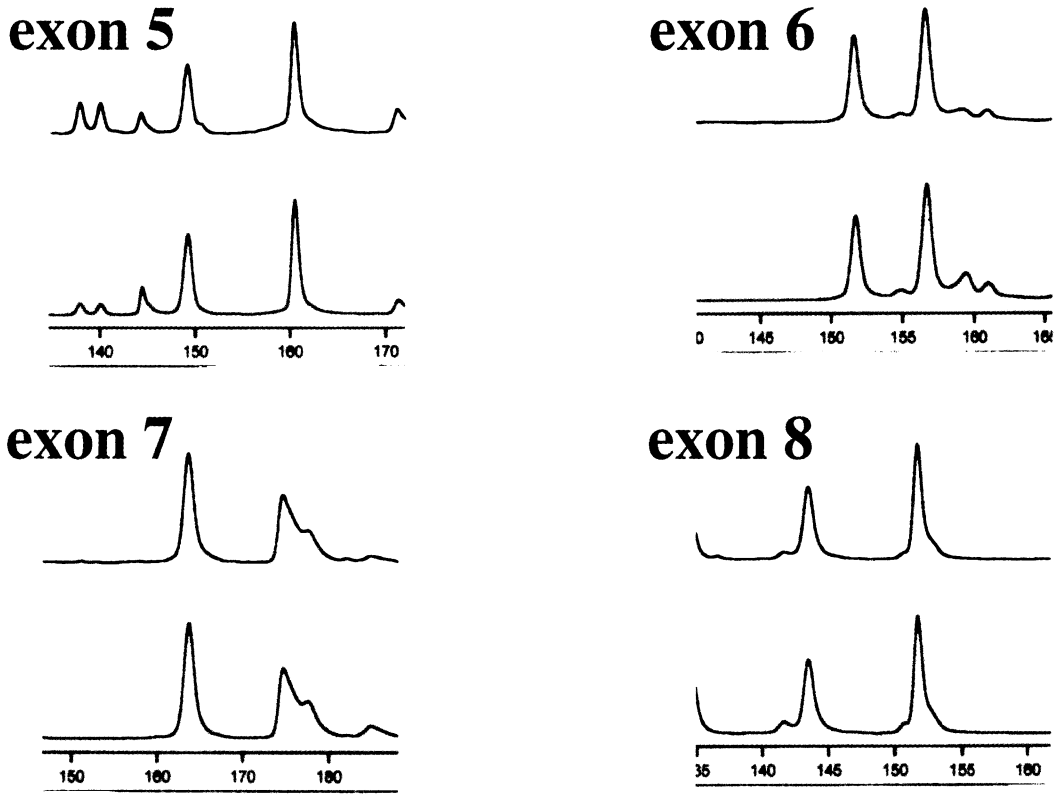

Figure 4. — PCR-SSCP analysis of the p53 gene (exons 5-8)
Single-stranded PCR products were analyzed by an auto-sequencer. No abnormal peak was detected. Upper: this case; Lower: wild-type p53 gene (control).

They used the term "trophoblastic pseudotumor" to reflect the clinically benign nature of this tumor. In 1981, Twiggs *et al.* reported the death of a patient with trophoblastic pseudotumor caused by metastasis [2]. Scully *et al.* then devised the term "placental site trophoblastic tumor" to reflect the malignant potential of this tumor [3].

PSTT is a neoplasm composed of a monomorphic population of intermediate trophoblasts. Histologically, PSTT is characterized by a mononuclear cell population that infiltrates the myometrium and its blood vessel walls. A dimorphic pattern of cytotrophoblasts and syncytiotrophoblasts is not seen. Villus formation is not observed and multinuclear giant cells are found occasionally [4]. Generally, hemorrhage and necrosis, which are usually associated with choriocarcinoma, are not observed. In the present case, however, these changes were widely observed, and might have been caused by chemotherapy with EMA/CO. Immunohistochemical staining was diffusely positive for hPL and only focally positive for hCG. Vardar *et al.* reported that 50~100% of intermediate trophoblasts stained for hPL, but that staining for hCG was positive in less than 10% of tumor cells in PSTT [5]. PSTT must be differentiated from choriocarcinoma and an exaggerated placental site. Compared with choriocarcinoma, PSTT has characteristic microscopic findings such as a monomorphic cell population, a pattern of immunohistochemical staining for hCG and hPL, and, generally, lack of necrosis and hemorrhage. PSTT can be also distinguished from an exaggerated placental site by lack of villus formation and occasional mitotic figures [6].

Sonographic findings in PSTT appear to be characteristic. In the present case, multiple cystic lesions of several sizes were identified in the tumor mass. These cystic lesions were considered to be dilated vessels since blood flow was evident in them on color flow doppler images. Kashimura *et al.* noted that perivascular invasion of neoplastic cells might cause these dilated vessels [7].

Unlike choriocarcinoma and other gestational trophoblastic tumors, PSTT is relatively unresponsive to chemotherapy. Therefore, despite aggressive chemotherapy, cases of metastasis (FIGO stage III~IV) of PSTT are unfortunately associated with a worse prognosis [8]. EMA/CO, originally described by Bagshawe [9], is one of the first-line chemotherapeutic regimens for high-risk gestational trophoblastic tumors. Swisher *et al.* found the total response rate to EMA/CO of cases of metastatic PSTT to be 71%, with a complete response rate of 28% [10]. However, our patient (with non-metastatic PSTT) was unresponsive to EMA/CO therapy. It has been reported that failure of EMA/CO indicates the aggressive nature of the tumor [11]. Furthermore, in the present case, although serum hCG was decreased after chemotherapy with EMA/CO, the uterine mass increased in size. Hopkins *et al.* suggested that the trophoblasts producing hCG were responsive to chemotherapeutic agents and that non-hCG-producing trophoblasts were resistant [12]. Thus, unlike gestational choriocarcinoma, the serum hCG level in patients with PSTT does not reflect the total tumor burden present. Therefore, caution is required when using serum hCG levels to evaluate therapeutic effects.

Many studies have examined the genetic and molecular events that occur in the development of malignant tumors. The p53 gene has been the most commonly studied as related to tumor malignancy. However, little is known concerning molecular changes of the p53 gene in PSTT. There have been a few reports concerning the relationship between p53 protein expression and PSTT [13,14]. However, these studies used only immunohistochemistry. The incidence and status of p53 gene mutations in PSTT have not been determined. In our case, immunohistochemical staining for p53 revealed intense nuclear labeling. Usually, but not always, the product of wild-type p53 is undetectable with immunohistochemical staining. We therefore performed PCR-SSCP analysis to determine whether p53 gene mutation was present in this case. However, no p53 gene mutation was detected in exons 5-8. Although overexpression on immunohistochemical staining suggested the presence of p53 mutation, some limitations of immunohistochemistry have been noted. Therefore, the relationship between p53 protein expression and p53 gene mutation is sometimes discrepant. Our findings suggest that p53 mutations may not play an important role in the development of PSTT. Analysis of more cases and other exons of the p53 gene will aid understanding of the pathogenesis of PSTT.

The clinical behavior of PSTT is still unclear and lacks reliable prognostic indicators. It is difficult to predict the clinical behavior of PSTT with serum hCG levels [4] and the mitotic count of the tumor cells [5,8]. In conclusion, immediate hysterectomy is required for patients without metastasis until the reliable prognostic indicators of PSTT are clearly understood.

References

[1] Kurman R. J., Scully R. E., Norris H. J.: "Trophoblastic pseudotumor of the uterus: an exaggerated form of 'syncytial endometritis' simulating a malignant tumor'. *Cancer*, 1976, *38*, 1214.

[2] Twiggs L. B., Okagaki T., Phillips G. L., Stroemer J. R., Adcock L. L.: "Trophoblastic pseudotumor-evidence of malignant disease potential". *Gynecol. Oncol.*, 1981, *12*, 238.

[3] Scully R. E., Young R. H.: "Trophoblastic pseudotumor: a reappraisal". *Am. J. Surg. Pathol.*, 1981, *5*, 75.

[4] Finkler N. J.: "Placental site trophoblastic tumor. Diagnosis, clinical behavior and treatment". *J. Reprod. Med.*, 1991, *36*, 27.

[5] Vardar M. A., Altintas A.: "Placental-site trophoblastic tumor. Principles of diagnosis, clinical behaviour and treatment". *Eur. J. Gynaecol. Oncol.*, 1995, *16*, 290.

[6] Motoyama T., Ohta T., Ajioka Y., Watanabe H.: "Neoplastic and non-neoplastic intermediate trophoblasts: an immunohistochemical and ultrastructural study". *Pathol. Int.*, 1994, *44*, 57.

[7] Kashimura M., Kashimura Y., Oikawa K., Sakamoto C., Matsuura Y., Nakamura S.: "Placental site trophoblastic tumor: immunohistochemical and nuclear DNA study". *Gynecol. Oncol.*, 1990, *38*, 262.

[8] Chang Y. L., Chang T. C., Hsueh S., Huang K. G., Wang P. N., Liu H. P., Soong Y. K.: "Prognostic factors and treatment for placental site trophoblastic tumor-report of 3 cases and analysis of 88 cases". *Gynecol. Oncol.*, 1999, *73*, 216.

[9] Bagshawe K. D.: "Treatment of high-risk choriocarcinoma". *J. Reprod. Med.*, 1984, *29*, 813.

[10] Swisher E., Drescher C. W.: "Metastatic placental site trophoblastic tumor: long-term remission in a patient treated with EMA/CO chemotherapy". *Gynecol. Oncol.*, 1998, *68*, 62.
[11] Janni W., Hantschmann P., Rehbock J., Braun S., Lochmueller E., Kindermann G.: "Successful treatment of malignant placental site trophoblastic tumor with combined cytostatic-surgical approach: case report and review of literature". *Gynecol. Oncol.*, 1999, *75*, 164.
[12] Hopkins M. P., Drescher C. W., McQuillan A., Keyser J., Schmidt R.: "Malignant placental site trophoblastic tumor associated with placental abruption, fetal distress, and elevated CA-125". *Gynecol. Oncol.*, 1992, *47*, 267.
[13] Muller-Hocker J., Obernitz N., Johannes A., Lohrs U.: "P53 gene product and EGF-receptor are highly expressed in placental site trophoblastic tumor". *Hum. Pathol.*, 1997, *28*, 1302.
[14] Ichikawa N., Zhai Y. L., Shiozawa T., Toki T., Noguchi H., Nikaido T., Fujii S.: "Immunohistochemical analysis of cell cycle regulatory gene products in normal trophoblast and placental site trophoblastic tumor". *Int. J. Gynecol. Pathol.*, 1998, *17*, 235.

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