An extragonadal yolk sac tumor presumed to be of postmeiotic germ cell origin by genetic zygosity analysis via single nucleotide polymorphism array

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BRIEF REPORT

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An extragonadal yolk sac tumor presumed to be of postmeiotic germ cell origin by genetic zygosity analysis via single nucleotide polymorphism array

Daisuke Tamura[1,2] | Daichi Maeda[3] | Toshiharu Sato[1] | Tae Sugawara[1] | Hiromitsu Shirasawa[1] | Dai Shimizu[1] | Naoki Sato[1] | Akiteru Goto[4] | Yukihiro Terada[1]

[1]Department of Obstetrics and Gynecology, Graduate School of Medicine, Akita University, Akita, Japan

[2]Department of Gynecology, Akita Red Cross Hospital, Akita, Japan

[3]Department of Clinical Genomics, Graduate School of Medicine, Osaka University, Osaka, Japan

[4]Department of Cellular and Organ Pathology, Graduate School of Medicine, Akita University, Akita, Japan

Correspondence
Daisuke Tamura, Department of Obstetrics and Gynecology, Graduate School of Medicine, Akita University, Akita, Japan, 1-1-1 Hondo, Akita-shi, Akita, 010-8543, Japan.
Email: tamurad@med.akita-u.ac.jp

Abstract

An extragonadal yolk sac tumor (YST) is a rare malignant germ cell tumor that usually occurs in childhood. The pathogenesis of extragonadal YST remains largely unknown, especially with regards to its cell of origin. Herein, we report a case of extragonadal YST arising in the uterine round ligament. A 31-year-old Japanese woman, para 2, underwent partial resection of a left-sided, 5-cm, solid inguinal mass. Intraoperative findings showed enlargement of the uterine round ligament in the inguinal canal. Pathological evaluation diagnosed the mass as YST with a mature teratoma (MT) component. The preoperative α-fetoprotein level was markedly elevated, at 24 790 ng/mL. Postoperative magnetic resonance imaging revealed a right ovarian MT and a 3-cm mass remaining in the left lower abdominal wall. The patient underwent total abdominal hysterectomy, bilateral adnexectomy, and left inguinal mass resection. We sampled three frozen tissues (YST, right ovarian MT, and left normal ovary) and performed a single nucleotide polymorphism (SNP) array. Pathological evaluation revealed remnant extragonadal YST in the left inguinal region. The SNP array demonstrated a completely homozygous YST genotype. Copy number variations were gains of 1p, 1q, 2p, 3p, 7p, 8p, 10q, 14q, 18p, 20q, Xp, and Xq and losses of 12q, 20p, and Xq. The right ovarian MT and left normal ovary were partially homozygous and heterozygous, respectively. The evidence suggests that this neoplasm is presumed to be a postmeiotic germ cell origin.

KEYWORDS

extragonadal germ cell tumor, genetic zygosity, single nucleotide polymorphism array, yolk sac tumor

1 | INTRODUCTION

Yolk sac tumor (YST), previously called endodermal sinus tumor, is a malignant germ cell tumor (GCT) that differentiates into somatic tissues such as yolk sac, intestinal tract, and liver, and produces α-fetoprotein (AFP). Malignant GCT accounts for about 5% of malignant ovarian tumor, and YST comprises approximately 1% of malignant ovarian tumor.[1] GCT arises mostly from the gonads, while extragonadal development occurs in approximately 10% of cases.[2] It has been reported that extragonadal YST occurs in the pineal body,

anterior mediastinum, sacrococcygeal region, uterus, vulva, retroperitoneum, and omentum.[1,2]

Teilum[3] proposed that GCT originates from primordial germ cells (PGCs). PGCs migrate from the yolk sac to the genital ridge via the hindgut during the embryonic period. Extragonadal development occurs predominantly in the midline, which is the migration pathway of PGCs. The source of extragonadal GCT is considered to be PGCs that are abnormally positioned due to a migration abnormality.[4] GCT has been examined based on genetic findings such as copy number variation (CNV) and zygosity. Oosterhuis et al.[5] classified GCT into five types according to anatomical site, phenotype, age, and genotype. CNVs in type 1 YST in prepuberty demonstrate gains of 1q, 12(p13), and 20q, and losses of 1p, 4, and 6q. Type 2 YST after puberty showed a characteristic gain of 12p.

The genetic zygosity of YST is not well understood. Ichikawa et al.[6] studied the zygosity of 10 ovarian YSTs in childhood using a single nucleotide polymorphism (SNP) array, and found that two YSTs were completely homozygous, six were partially homozygous, and two were heterozygous. They also examined the zygosity of five childhood extragonadal YSTs, including two in the sacrococcygeal region, one in the mediastinum, one in the retroperitoneum, and one in the stomach; of these, three were partially homozygous and two were heterozygous. Genetic findings of childhood gonadal and extragonadal YST have accumulated recently, but very few studies have investigated CNVs and zygosity in extragonadal YST in adulthood. Therefore, we sampled frozen tissues from extragonadal YSTs arising in the uterine round ligament and performed SNP array analysis, comparing the results of YST with those of right ovarian mature teratoma (MT) and left normal ovary.

2 | METHODS AND RESULTS

2.1 | Patient

A 31-year-old Japanese woman (para 2) had visited another clinic 6 months earlier due to a left inguinal mass that had been increasing in size. She had no cancer history. Computed tomography (CT) revealed a 5-cm right ovarian tumor and an internal heterogeneous solid mass of approximately 5-cm in the left inguinal region (Figure S1A,B). Her preoperative AFP level was markedly elevated at 24 790 ng/mL. A first surgery was performed due to the preoperative diagnosis of left inguinal tumor. Intraoperative findings when the left inguinal canal was released revealed enlargement of the left round ligament into a solid mass that was firmly attached to the surrounding tissues. Complete removal of the left inguinal tumor was not possible, so partial resection was performed. She was diagnosed as YST with a MT component based on histopathological evaluation, and was admitted to our hospital for further examination and treatment. Magnetic resonance imaging revealed a right ovarian tumor and a multilocular mass about 3-cm in size that remained beaded below the left inguinal abdominal wall (Figure S1C-E). The AFP value at her first visit to our

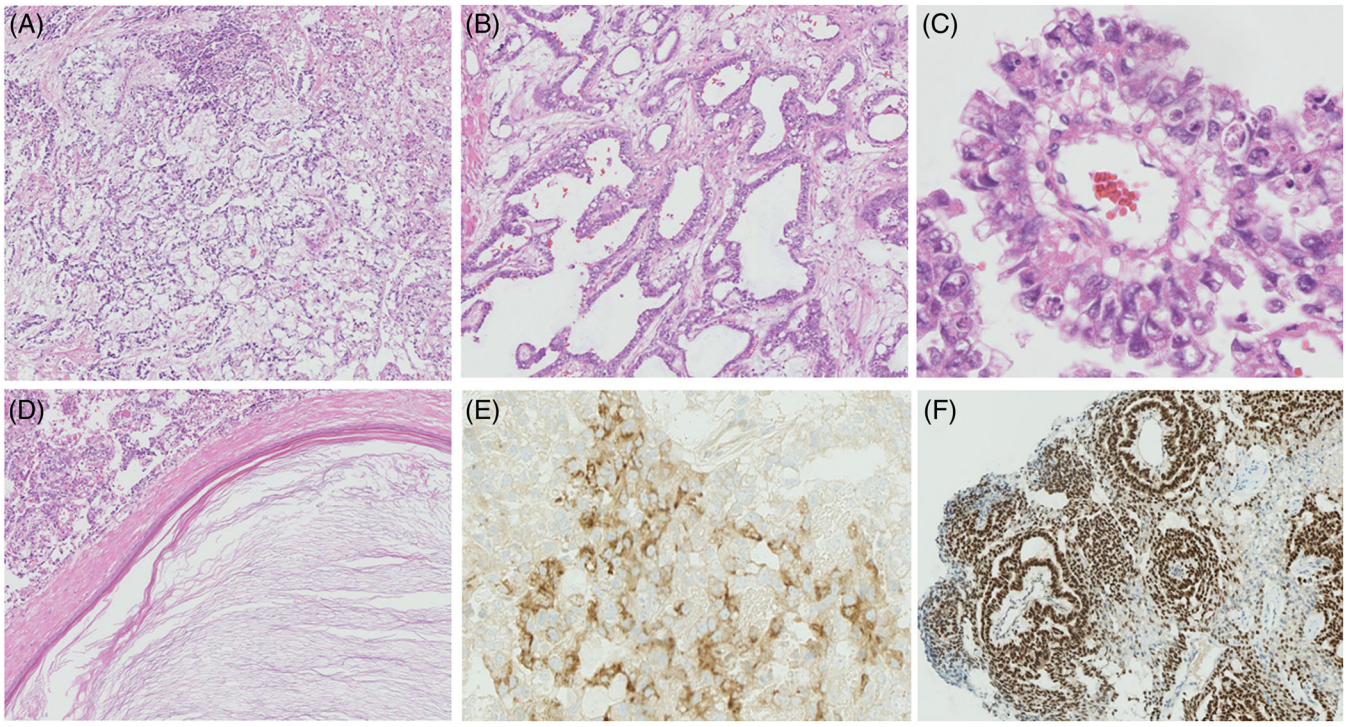

FIGURE 1 A, YST component showing reticular pattern of growth. B, An area of YST component where glandular structures are predominant. C, Schiller-Duval body. The tumor cells have nuclei with coarse chromatin. Moderate cytological atypia is observed. D, MT component composed of keratinized stratified squamous epithelium without atypia. E, Positive AFP immunoreactivity in YST component. F, Diffuse nuclear SALL4 positivity in tumor cells of YST [Color figure can be viewed at wileyonlinelibrary.com]

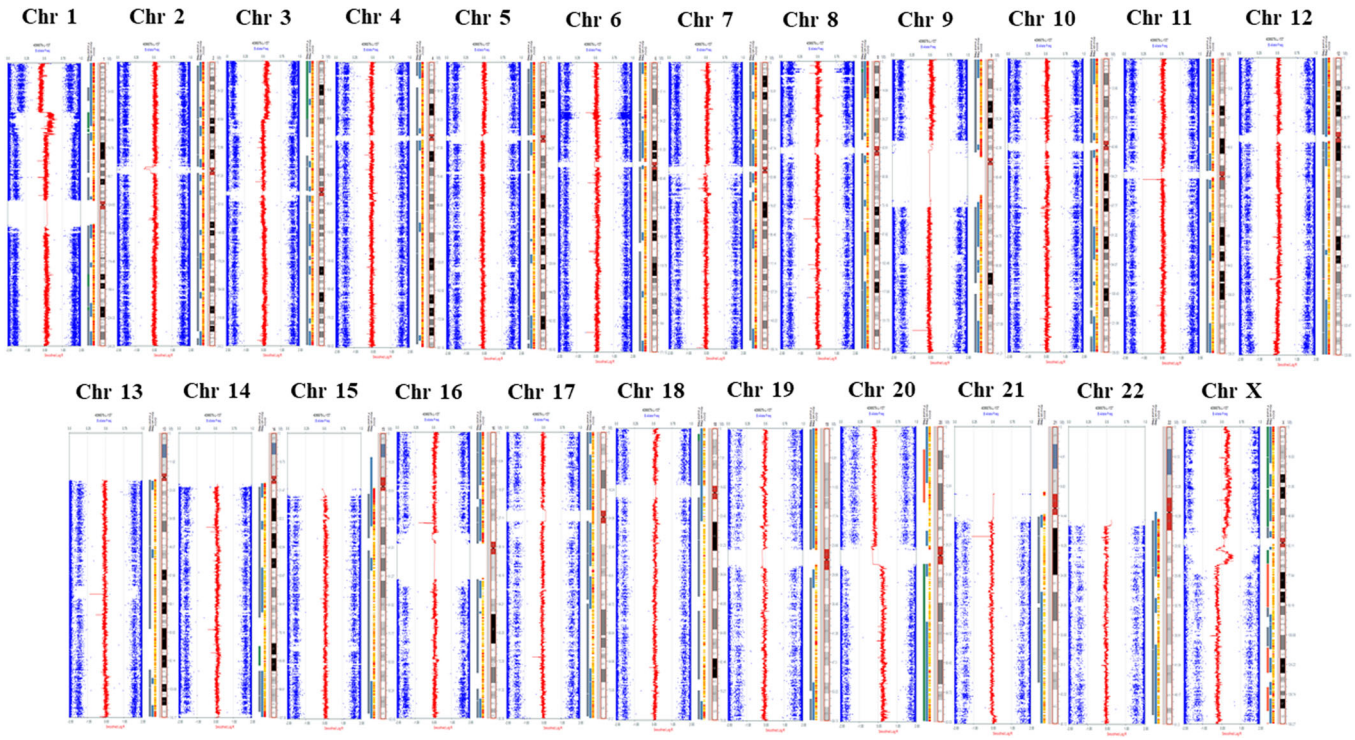

FIGURE 2 SNP array analysis of autosomal and sex chromosomes in extragonadal YST. Red line indicates smoothed log R ratio, which specifies gain or loss. Blue dots indicate B-allele frequency (BAF), which represents the fluorescence intensity of SNP alleles. The loss of heterozygosity (LOH), CNV, and genomic positions are indicated below the ideograms, with red point representing the centromere. Grey, green, and orange bars indicate copy-neutral LOH, copy number gain, and copy number loss, respectively. Because BAF plots at nearly 0 or 1 value in all chromosomes, this extragonadal YST is shown to be completely homozygous [Color figure can be viewed at wileyonlinelibrary.com]

hospital was 877 ng/mL. She underwent abdominal hysterectomy, bilateral adnexectomy, and inguinal tumor resection, because she did not hope to maintain fertility preservation and the primary site of the YST was unknown. Pathological evaluation revealed the presence of remnant YST confined to the left inguinal region. MT was identified in the right ovary. No tumor was found in the left adnexa or uterus. The remnant YST in the left inguinal mass was involved in the left uterine round ligament in the inguinal canal; therefore, the final diagnosis was extragonadal YST originating from the left uterine round ligament. She received BEP therapy (bleomycin, etoposide, and cisplatin) as adjuvant chemotherapy. At the end of three cycles of BEP therapy, the AFP level decreased to 17.8 ng/mL (Figure S2). Because of concerns about interstitial pneumonia caused by bleomycin, one cycle of EP therapy (etoposide and cisplatin) was added, and this caused the AFP level to reach the normal range. She has remained free of disease for more than 4 years after chemotherapy.

2.2 | Pathological features

The left inguinal mass that was resected during the first surgery consisted of YST and MT components. The YST component comprised tumor cells with hyperchromatic nuclei that grew predominantly in reticular, glandular, and solid patterns. Moderate nuclear atypia was observed. There were areas with abundant hyaline globules. Prominent edema was observed in some areas, as shown in Figure 1A-D. Immunohistochemically, the tumor cells were positive for cytokeratin (AE1/AE3), vimentin, AFP, glypican-3, placental alkaline phosphatase, and SALL4 and negative for CD30, estrogen receptor, and progesterone receptor (Figure 1E,F). The area of MT was very focal and consisted of cystic cavities lined by mature keratinising squamous epithelium. The left inguinal YST that was resected in the second surgery showed morphological features similar to those of the initially resected tumor.

2.3 | Single nucleotide polymorphism array analysis

Fresh frozen specimens (left inguinal YST, right ovarian MT, and left normal ovary) were obtained from the patient after provision of informed consent and approval by the Akita University ethics committee. Tumor specimens used in the analysis were stained with hematoxylin and eosin and confirmed to have tumor contents of over 80%. DNA extraction was performed using the phenol-chloroform extraction method.[7] SNP array analysis was performed using Illumina HumanOmniExpress Exome-8 v1.2 containing 958 497 markers. Allele ratios and intensity information were analyzed in terms of CNVs

using KaryoStudio v1.4 and Illumina GenomeStudio v2011.1, as previously reported.[8] Genomic positions are based on the GRCh37/hg19 human genome assembly.

Intriguingly, all chromosomes in the extragonadal YST demonstrated complete homozygosity, as shown in Figure 2. Furthermore, the CNVs were gains of 1p, 1q, 2p, 3p, 7p, 8p, 10q, 14q, 18p, 20q, Xp, and Xq and losses of 12q, 20p, and Xq. On the other hand, the right ovarian MT and left normal ovary were partially homozygous and heterozygous, respectively (Figures S3 andS4).

3 | DISCUSSION

In this study, we demonstrated for the first time the complete homozygosity of extragonadal YST with a MT component in adulthood. To date, there have been numerous reports on the close relationship between MT and YST. Yoshida et al.[9] reported that 13 of 241 patients (5.4%) developed YST after pediatric sacrococcygeal teratoma resection. Utsuki et al.[10] described a case in which YST occurred after intracranial MT resection. Based on pathological findings, the authors suspected malignant transformation from MT to YST. Ichikawa et al.[6] analyzed the methylation status of 15 tumor suppressor genes and two imprinted genes, SNPPN and H19, in 28 children with GCTs (MT, immature teratoma, and YST). They found frequent methylation in YST, and concluded that this may be a sign of malignant transformation of MT to YST. The patient in our case had extragonadal GCTs that pathologically demonstrated primarily YST components with a focal MT component, which may be consistent with the theory of malignant transformation to YST.

Extragonadal GCT is thought to be caused by abnormal migration of PGC remnants from the yolk sac to the genital ridge.[4] Through meiotic cell division, heterozygous PGCs develop into homozygous postmeiotic germ cells.[11] Several studies[12,13] thus far have investigated zygosity in MT. Surti et al.[14] proposed five mechanisms of ovarian teratoma development, according to chromosomal variants of the centromeric region and distal polymorphic DNA markers. Endoreduplication indicates that the entire gene region, including the centromere, is homozygous. Our extragonadal YST showed complete homozygosity in all examined chromosomal regions (not including the centromere), suggesting that this YST was of PGC origin and was derived from endoreduplication, with a MT background.

Ravishankar et al.[2] reviewed the pathological and clinical characteristics of 49 extragonadal YSTs, including 15 cases at their institution. They hypothesized that menopausal or late menopausal extragonadal YST is a somatic tumor or differentiated YST, and true germline extragonadal YST in young patients may respond to chemotherapy, whereas the efficacy of chemotherapy on somatically derived extragonadal YSTs is unclear. McNamee et al.[15] proposed the term “somatically derived YST” to indicate YST-like neoplasms derived from somatic cells older than 40 years. Examining zygosity with SNP arrays may help to determine whether YSTs are of somatic or germ cell origin. Our patient responded well to chemotherapy and has remained disease-free for more than 4 years. This clinical course and the complete homozygosity of her extragonadal YST are compatible with the theory that this YST was derived from a true GCT.

In conclusion, we report a case of extragonadal YST with a MT component arising in the uterine round ligament with a successful response to chemotherapy. We used SNP array analysis to demonstrate for the first time the complete homozygosity of an extragonadal YST in adulthood. This genotype suggests that this neoplasm is presumed to be a postmeiotic germ cell origin.

CONFLICT OF INTEREST

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AUTHOR CONTRIBUTIONS

D.T. and D.M. performed the research. D.T., D.M., T.S., T.S., H.S., D.S., N.S., A.G., and Y.T. provided essential tools. D.S. and D.T. performed surgery. D.T. and D.M. wrote the paper. All the authors approved the final manuscript.

ETHICS STATEMENT

Ethical approval was obtained from Akita University, Faculty of Medicine, Ethics Committee (Reference Nos. 1211 and 1246).

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

ORCID

Daisuke Tamura https://orcid.org/0000-0001-9808-1897

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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