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BRIEF REPORT

Ovarian germ cell tumor/mastocytosis with *KIT* mutation: A unique clinicopathological entity

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Abstract

Most tumors are sporadic and originated from somatic mutations. Some rare germline mutations cause familial tumors, often involving multiple tissues or organs. Tumors from somatic mosaicism during embryonic development are extremely rare. We describe here a pediatric patient who developed both an ovarian germ cell tumor and systemic mastocytosis. Targeted DNA next-generation sequencing analysis revealed similar genomic changes including the same *KIT* D816V mutation in both tissues, suggesting a common progenitor cancer cell. The *KIT* mutated cells are likely from early embryonic development during germ cell migration. A literature search found additional eight similar cases. These diseases are characterized by pediatric-onset, all-female, neoplastic proliferation in both gonad and bone marrow, and a common oncogenic cause, that is, *KIT* mutation, constituting a clinically and genetically homogenous disease entity. Importantly, the association of germ cell tumors with hematopoietic neoplasms suggests that the primordial germ cells are the primitive hematopoietic stem cells, a much-debated and unsettled question.

KEYWORDS
germ cell tumors, *KIT*, mastocytosis

1 | INTRODUCTION

One of the fundamental questions in biology is the origin of hematopoietic stem cells. Previous data suggest that the aorta in the aorta-gonad-mesonephros (AGM) region is the site of the first definitive hematopoiesis during embryo development of mammalian[1,2]; however, the primitive precursor for definitive hemopoiesis remain inconclusive. Studies of human embryonic hematopoiesis are particularly challenging due to ethical considerations. Human diseases caused by early embryonic errors, therefore, provide a unique opportunity for us to understand the complex embryonic development including early hematopoiesis. We describe herein a rare case of ovarian germ cell tumor with concurrent systemic mastocytosis. Both tumors carried two copies of *KIT* D816V mutation caused by copy neutral loss of heterozygosity (CN-LOH) of chromosome 4, suggesting that these tumors are originated from the same mutated primordial germ cells (PGCs). Our case is the ninth case of ovarian germ cell tumor with mastocytosis, which likely constitutes a unique disease entity, we termed ovarian germ cell tumor/mastocytosis with *KIT* mutation, in line with the WHO classification of tumors by using both histological and molecular features. These diseases are characterized by pediatric-onset, all-female, tumors in both gonad and bone marrow, and a common oncogenic cause, that is, *KIT* mutation. Importantly, the association of germ cell tumors and hematopoietic neoplasms not only indicates shared progenitor cancer cells but also suggests that the PGCs are indeed the primitive hematopoietic stem cells.

Peifang Xiao and Ping Chen contributed equally to this study.

2 | MATERIALS AND METHODS

2.1 | Karyotype

Cells from bone marrow aspirate in sodium heparin were counted with a TC-20 Automated Cell Counter (Bio-Rad, Hercules, CA). The 5×10^6 of cells were cultured in Marrow Max (Life Technologies, Carlsbad, CA) overnight. Cells were treated with ethidium bromide (5 μg/ml) for 1.5 h and colcemid (0.5 μg/ml) for 20 min, incubated in a hypotonic solution, fixed, and metaphase spreading slides were manually made. After baking at 70°C for 1 h, chromosomes were Giemsa-Trypsin banded and analyzed.

2.2 | Immunohistochemical studies

Immunohistochemistry (IHC) were performed on 5-μm tissue sections. The slides were baked at 60°C for 1 h; deparaffinized and rehydrated with 100% xylene, 100%/70%/50% ethanol, and running water; and blocked in 10% normal serum with 1% bovine serum albumin (BSA) in Tris-buffered saline. The slides were incubated with primary antibodies for 2 h, blocked of endogenous peroxidase with 0.3% hydrogen peroxidase, and incubated in horseradish peroxidase-labeled polymer (DAKO) according to the manufacturer's instructions. The tissue sections were developed using 3,3′-diaminobenzidine as the chromogen (DAKO) and counterstained with Mayer hematoxylin.

2.3 | Targeted DNA next-generation sequencing

DNA was extracted from bone marrow aspirate and 10-μm tissue sections with a standard proteinase K and SDS protocol. DNA was fragmented with a Bioruptor Pico (Diagenode, Denville, NJ) to 200–300 bp, subjected to end-polishing, phosphorylation, and dA extension by incubating with the end-repair mix, Klenow exo- and Taq polymerase (Enzymatics, Beverly, MA) for 15 min at 12°C, 15 min at 37°C, and 15 min at 72°C and ligated to a UMI-containing adaptor. Four cycles of polymerase chain reaction (PCR) were performed with adaptor-specific primers and the PCR products were incubated with a pool of biotin-labeled bait oligos targeted 638 genes commonly involved in tumor for 16 h. Targeted regions were enriched by pull-down with streptavidin beads, amplified by PCR, and sequenced in an

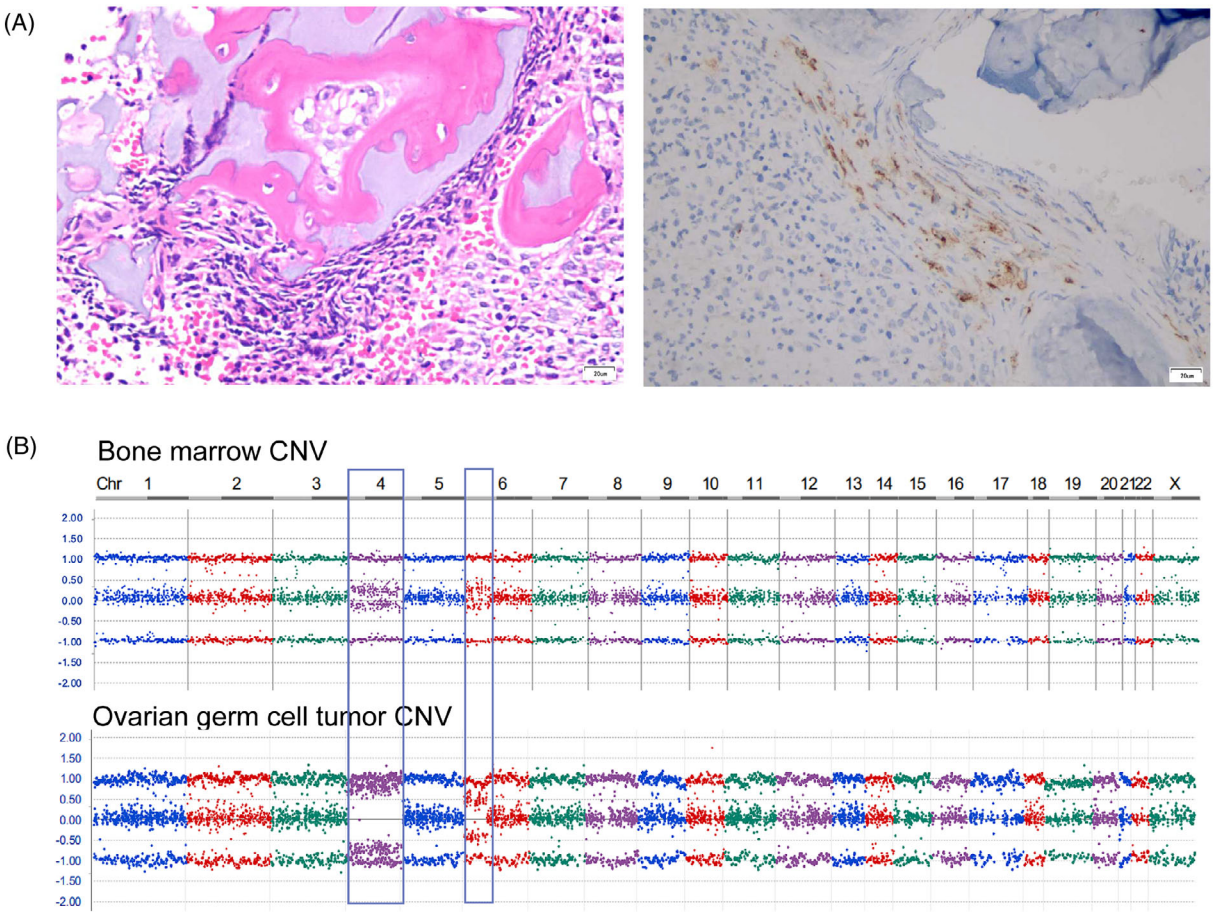

FIGURE 1 (A) Bone marrow biopsy showed densely packed, spindled mast cells in paratrabecular area (left panel) and were positive for CD117 stain, consistent with systemic mastocytosis; (B) both bone marrow (top panel) and ovarian germ cell tumor had copy neutral loss of heterozygosity of chromosomes 4 and 6p, consistent with a same progenitor cancer cell

TABLE 1 Clinical and mutation information of nine cases of ovarian germ cell tumor/mastocytosis with *KIT* mutation

Gonadal tumor	Ages/sex	Time to SM	Mutated genes in GCT	Mutated genes in SM	References
Teratoma	3/F	1 m	*KIT* D816V	*KIT* D816V	3,4
Mixed GCT	16/F	2 m	*KIT* N822K (hmz)	*KIT* N822K (hmz)	4
Mixed GCT	13/F	2 m	*KIT* D816H, i(12p)	*KIT* D816H, i(12p)	5
Dysgerminoma	13/F	24 m	*KIT* D816A	*KIT* D816A *TP53* E62X	6
Mixed GCT	3/F	1 m	*KIT* D816V (hmz) *MTOR* L2220F *TP53* L265P	*KIT* D816V (hmz) *MTOR* L2220F	This study
Mixed GCT	18/F	36 m	N/D	N/D	7
Mixed GCT	12/F	6 m	N/D	N/D	8
Mixed GCT	10/F	3 m	N/D	*KIT* D816, V560 negative	9
Teratoma	6/F	12 m	N/D	*KIT* D816V negative	10

Illumina NextSeq sequencer. Sequencing results were analyzed with SeqNext software (JSI, Ettenheim, Germany).

This study is approved by the institutional review board at respective institutions.

3 | RESULTS

3.1 | Case report

A 3 1/2-year-old girl presented with a painless left abdominal mass. Ultrasonography found a solid mass of 8.8 × 5.7 × 8.4 cm. Blood chemistry showed increased serum alpha-fetoprotein (AFP) at 19, 900 ng/ml (normal range 0–20 ng/ml) and human chorionic gonadotropin (β-hCG) at 68.02 mIU/ml (normal range < 11.6 mIU/ml). The tumor was surgically removed, which contained both cystic and solid/fleshy areas. Histopathology found cartilage, neuroectodermal elements including choroid plexus and glial cells, dysgerminoma, and yolk sac tumor components. IHC showed CK+, EMA+, AFP+, OCT3/4+, SALL4+, CD30−, GPC-3+,S100 +, PHOX2B-, SYN+, CD117+, PGP9.5+, NSE+, PLAP+, Ki67 (60%). A final diagnosis of ovarian mixed germ cell tumor, Stage 2, was made and six cycles of adjuvant chemotherapy with a combination of vindesine, ifosfamide, and etoposide were performed and the patient recovered well, with normal AFP (1.75 ng/ml) and normal β-hCG (0.01 mIU/ml) at the end of chemotherapy. One month after the end of chemotherapy, peripheral blood counts demonstrated leukocytosis with WBC 39.01 × 10^9/L, Hb 124 g/L, platelet count 208 × 10^9/L, RBC 5.00 × 10^{12}/L, neutrophile 31.60 × 10^9/L, and lymphocytes 3.04 × 10^9/L. Ultrasonography found an enlarged spleen and liver (3.6 cm and 3.1 cm below the costal margin, respectively). Bone marrow biopsy showed marked granulocytic proliferation, with a myeloid-to-erythroid ratio of 38:1. Myeloblasts and promyelocytes were not increased, and no myeloid dysplasia was present. Erythroid and lymphoid elements were markedly proportionally decreased with no significant dysplasia. Loose clusters of megakaryocytes were observed with some small hyposegmented megakaryocytes seen. Densely packed, spindled mast cells were noted in paratrabecular area and were stained positive for CD117 (Figure 1A). Chromosome analysis of bone marrow aspirates showed a normal female karyotype (46,XX). A targeted DNA NGS assay showed *KIT* D816V (VAF 13.9%), *MTOR* L2220F (VAF 9.7%), and copy neutral loss of heterozygosity (CN-LOH) for chromosomes 4 and 6p (Figure 1B). The patient was diagnosed with systemic mastocytosis with an associated hematological neoplasm (AHN), treated with hydroxycarbamide, and is now in the process of bone marrow transplantation. A retrospective targeted DNA NGS assay on the patient's prior ovarian mixed germ cell tumor showed an identical *KIT* D816V (VAF 74.1%), *MTOR* L2220F (VAF 29.1%), CN-LOH of 4 and 6p, and an additional *TP53* L265P (VAF 9.4%) (Figure 1B).

4 | DISCUSSION

Because the systemic mastocytosis occurred just 1 month after chemotherapy for the patient's ovarian mixed germ cell tumor, a therapy-related hematological disease was ruled out. These two otherwise histologically unrelated tumors carried the same neoplastic clone, consistent with a common primitive cancer cell. A literature search found eight additional similar cases (Table 1).[3–10] These patients are all females with a pediatric-onset and a median age of 10 years (range 3–18 years). The types of gonadal tumors include mixed germ cell tumor (six cases), teratoma (two cases), and dysgerminoma (one case). All patients had mastocytosis; three of them had a cutaneous presentation. Five of nine cases had a *KIT* mutation, with four of them being the hot spot D816 mutation. Two of the *KIT*-mutated cases had CN-LOH, leading to a doubled dose of mutated-*KIT*. The remaining four cases had no or incomplete genetic testing. Because of the marked similarity in clinical presentation and the common *KIT* mutation, we propose to classify these diseases as one disease entity, that is, ovarian germ cell tumor/mastocytosis with *KIT* mutation.

Another type of germ cell tumor, mediastinal germ cell tumor, is also associated with hematopoietic neoplasms; however, these tumors do not harbor *KIT* mutation, instead, have heterogeneous genomic alterations including isochromosome 12p and mutated *TP53*, *RAS*,

and/or *PTEN*. PGCs with complex genetic changes are suspected to be inefficient in migration; and therefore, lodge in the midline region and form mediastinal germ cell tumors. These patients develop different kinds of hematopoietic malignancy, including acute myeloid leukemia (AML), myelodysplastic syndromes (MDS), histiocytic sarcoma, and chronic myelomonocytic leukemia (CMML). Similar to our case, a common malignant clone was observed between mediastinal germ cell tumor and hematopoietic neoplasms, consistent with a shared malignant precursor.[11]

The *KIT* mutation is a hallmark of mastocytosis and is found in >90% of systemic mastocytosis. The *KIT* mutation is also seen in approximately one-third of dysgerminomas or seminoma, suggesting a critical role of KIT signaling in the development of germ cell tumors.[12] Why most dysgerminomas or seminoma with the *KIT* mutation do not develop hematopoietic neoplasms is unknown. One possibility is that most *KIT* mutation is acquired at a later stage of development, for example, after the completion of PGC migration. Consistently, the median age for dysgerminomas and seminoma is 22 years (range 11–65 years) and 41 years (range 34–47 years), both significantly older than our patients (median age of 10).[13] Therefore, it is likely the *KIT* mutation in dysgerminomas and seminoma occurs in gonocytes, instead of the migrating PGCs, when the gonocytes are actively dividing in the gonads.

PGCs are crucial for species conservation and are one of the first cell lineages isolated/specified at early embryonic development. PGCs need to migrate before setting in the gonads, and the migration route is species specific. In mice, PGC migration starts at epiblast and moves through extraembryonic endoderm, primitive streak, hindgut epithelium, medial mesentery, and reaches their destination at gonadal ridges. Mammalian PGCs are actively proliferating during their migration. In mice, approximately 45 PGCs were seen at epiblast, and the number of PGCs reaches 25 000 when the migration completes.[14,15] Cell proliferation is a precise but error-prone cellular process. Mutated cells are incapable of finishing the migration and will be eventually eliminated by apoptosis[16,17]; therefore, PGC migration is considered a quality control program for healthy germ cells.[18] KIT signaling plays a major role in the migration, proliferation, and survival of PGCs,[19–21] for example, apoptosis occurs when the Steel factor (KIT ligand) was down-regulated. PGCs with a *KIT* mutation achieve a ligand-independent KIT activation, leading to unimpacted migration. In contrast, PGCs with non-*KIT* mutations are likely unresponsive to migration cues and remain in the midline region; these dislocated PGCs are typically programmed to death; however, if the mutated cells are also deficient in apoptosis, they may survive to form tumors. Indeed, most of the mediastinal germ cell tumors contained mutations of *TP53* and/or *RAS*, with their altered signaling known to cause defective apoptosis.[11]

In conclusion, we described a unique case of ovarian germ cell tumor and systemic mastocytosis. By summarizing additional eight similar cases in the literature, we suggested that these diseases likely constitute a clinically and genetically homogenous disease entity, that is, ovarian germ cell tumor/mastocytosis with *KIT* mutation. Importantly, a single common mutated cell for both germ cell tumor and mastocytosis strongly supports the notion that the primitive precursors of definitive hematopoietic stem cells are primordial germ cells.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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