TP53 and PTEN Mutations Were Shared in Concurrent Germ Cell Tumor and Acute Megakaryoblastic Leukemia

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Abstract

Background: The occurrence of a mediastinal germ cell tumor (GCT) and hematological malignancy in the same patient is very rare. Due to its rarity, there have been only two reports of the concurrent cases undergoing detailed genetic analysis with whole-exome sequencing (WES), and the possible clonal relationship between the both tumors remained not fully elucidated. Methods: We performed whole-exome sequencing analysis of mediastinal GCT and acute myeloid leukemia (AML) samples obtained from one young male adult patient with concurrent both tumors, and investigated the possible clonal relationship between them. Results: 16 somatic mutations were detected in the mediastinal GCT sample and 18 somatic mutations in the AML sample. Mutations in nine genes, including TP53 and PTEN both known as tumor suppressor genes, were shared in both tumors. Conclusions: All in our case and in the previous two cases with concurrent mediastinal GCT and AML undergoing with whole-exome sequencing analysis, TP53 and PTEN mutations were commonly shared in both tumors. These data not only suggest that these tumors share a common founding clone, but also indicate that associated mediastinal GCT and AML harboring TP53 and PTEN mutations represent a unique biological entity.

Background

Germ cell tumors (GCTs) are the most common malignant tumors in adolescent males. About 2-5% of GCTs arise at extragonadal sites[1]. Among them, mediastinal GCTs (mGCTs) predominantly occur within the anterior mediastinum. Though mGCTs have different clinical characteristics from testicular GCTs, those were thought to be derived from gonadal lesions as there was no cytogenetic difference between them[2]. Since 1985, the unique and rare associations between hematological malignancies (HMs) and mGCTs were reported in approximately 60 cases[3, 4]. In most cases, the involved GCT is non-seminomatous and mediastinal, and the HM is acute myeloid leukemia (AML), frequently acute megakaryoblastic leukemia (AMKL) under the WHO 2017 classification, corresponding to AML M7 under the former French-American-British classification, and associations with myelodysplastic syndrome (MDS), myelomonocytic leukemia, and essential thrombocythemia have also been reported[4, 5]. The interval between the onset of mGCTs and that of HMs is occasionally <6 months, and the synchronous presentation of the two diseases is sometimes observed. HMs associated with mGCTs should be separated from therapy-related, secondary AML or MDS, which typically develop at least a year following exposure to cytotoxic drugs administered for GCT treatment. The association of HMs with mGCTs is extremely rare. In an large, international, multicenter database study of 635 extragonadal GCT patients, HMs were observed in 17 extragonadal GCTs[5]. All cases developed among the 287 mGCTs, giving an incidence rate in this group of 6%. The frequent presence of isochromosome 12p in AML samples from these patients strongly suggested that HMs and mGCTs might arise from common progenitor cells, because isochromosome 12p is the most common chromosomal abnormality in GCTs but is exceptionally rare in AML without mGCT association[5-8]. The recent discovery of *TP53* and *PTEN* mutation both in AML and mGCT in each one patient from 2 independent report not only strengthen the concept of the common progenitor cells, but also provide the molecular aspects for this unique and rare

association[9, 10]. Herein, we report a third case of the concurrent occurrence of mediastinal GCT and AML M7, in which we performed whole-exome sequencing (WES) analysis of both tumors and investigated the possible clonal relationship between them.

Methods

Sample collection

This study was approved by the Research Ethics Committee of the Faculty of Medicine, University of Miyazaki. GCT samples (the left cervical mass) and AML samples (bone marrow) were obtained from the patient with written content.

Cytogenetic analysis

Cytogenetic analysis of GCT and AML samples were performed by G-banding.

DNA extraction and whole-exome sequencing analysis

Genomic DNA of GCT samples and AML samples was extracted using the QIAamp DNA Mini kit. WES analysis of GCT and AML samples were performed, using his buccal mucosa as a germline control, as previously described [11]. SureSelect Human All Exon v6 kits (Agilent Technologies) were used for exome capture according to the manufacturer's instructions. Sequencing data were generated using the Illumina NextSeq 500 platform with a standard 150-bp paired-end read protocol, as previously described[11]. Sequence alignment and mutation calling were performed using the Genomon pipeline (https://github.com/Genomon-Project), as previously described. Putative somatic mutations with (i) Fisher's exact *P* value < 0.01; (ii) > 2 variant reads in tumor; (iii) allele frequency in tumor >= 0.035; (iv) allele frequency in germline < 0.035 were adopted and filtered by excluding (i) synonymous SNVs; (ii) variants only present in unidirectional reads; and (iii) variants occurring in repetitive genomic regions. These candidate mutations were further filtered by removing known variants listed in NCBI dbSNP build 131, the 1000 Genomes Project (October 2014 release), National Heart, Lung, and Blood Institute (NHLBI) Exome Sequencing Project (ESP) 6500, and the Human Genome Variation Database, unless they were listed in the COSMIC database (v70). Finally, all detected mutations were manually checked by Integrative Genomics Viewer (IGV) and their allele frequencies were calculated using pysam's pileup function (version 0.14.1).

Results

Clinical and pathological findings

The patient was a 37-year-old, previously healthy male who presented with a dry cough. He first admitted to his family doctor and was pointed out to have a 5 cm diameter left cervical tumor, then was referred to

our hospital. Examination revealed tachycardia (107/min) and elastic hard left cervical mass with a 5 cm diameter. A chest X-ray revealed a well-circumscribed bilateral hilar mass with a maximum dimension of 20.5 cm, and dullness of the right costal pleural angle (Figure 1A). Peripheral blood examination showed the followings: Hb level 16.2 g/dL; leucocyte count 9.9 x 10^9/L, and platelet count 293 x 10^9/L. Serum alpha-fetoprotein (AFP), beta-human chorionic gonadotropin (bhCG), and lactate dehydrogenase levels were 1921 ng/mL, 511 mIU/mL, and 390 IU/L, respectively. A computed tomography (CT) scan revealed a 19.5 cm x 10.8 cm heterogeneously enhancing anterior mediastinal mass and a 4.3 cm left cervical mass (Figure 1B). A surgical biopsy of the left cervical mass showed heterogenous features including immature cartilages, immature mesenchymal cells, columnar epithelium cells and yolk sac tumor-like components (Figure 2A, B). Immunohistochemical staining of these tumor cells revealed immunoreactivity with AFP and Glypican-3 (Figure 2C, D). He was diagnosed with non-seminomatous GCT, and was treated with BEP therapy (bleomycin, etoposide, and cisplatin). After starting the therapy, the serum bhCG level promptly decreased, but there was no reduction in the size of the mediastinal mass. Thrombocytopenia started 15 days after BEP therapy and persisted for 1 week. To evaluate its cause, bone marrow (BM) examination was performed. The BM aspirate showed that 74% of all nucleated cells were blasts, which were medium to large in size with round nuclei, and one to three nucleoli (Figure 2E). These cells were negative for myeloperoxidase by immunostaining (Figure 2F), and were positive for CD7 (79.6%), CD13 (82.6%), CD33 (81.1%), CD34 (99.1%), CD41a (99.1%) and CD117 (44.5%) by flow cytometry. BM biopsy showed hypercellular marrow, and blasts were positive for von Willebrand factor (Figure 2G, H). The cause of cytopenia was revealed to be AMKL. Induction chemotherapy with idarubicin and cytosine arabinoside was administered for AMKL. He achieved first complete remission with enough platelet recovery. The chemotherapy for AML had no effect on the GCT, and the mediastinal mass enlarged. We therefore continued therapy for GCT, 2 courses of TIP (paclitaxel, ifosfamide, and cisplatin), 1 course of TGO (paclitaxel, gemcitabine, oxaliplatin), and finally another course of BEP therapy. These treatments did not reduce the size of the mediastinal or cervical masses. AMKL relapsed during the TIP therapy for GCT, and thrombocytopenia, which required platelet transfusion every other day, continued during the therapy. Despite these treatments, he died 6 months after his initial diagnosis.

Cytogenetic analysis and whole-exome sequencing analysis

To clarify the possible clonal relationship between the GCT and AML, we performed cytogenetic and WES analysis of GCT and AML samples. In the cytogenetic analysis, the AML sample revealed a hyperdiploid karyotype: 63XXY,+Y,+1,-2,-4,-5,add(6)(p21),+8,-9,-11,-13,-17,-18,-19 in 4/20 metaphases and 46XY in 16/20 metaphases; while, no analyzable metaphases were obtained in the GCT sample. Therefore, cytogenetic analysis did not provide us enough information to clarify the clonal relationship between both tumors. In the WES analysis, we detected 16 somatic mutations in the GCT sample, including 15 single nucleotide variants (SNVs) and one deletion, and 18 somatic mutations in the AML samples, including 17 SNVs and one deletion. Among them, mutations in 9 genes, specifically *TP53, PTEN*, *RLF, DLG2, YY2, PCLO, GOLGA8J, EDRF1,* and *ASF1A* were observed in both tumors; and *TP53, PTEN*, *RLF, DLG2,* and *YY2* showed relatively higher variant allele frequencies than *PCLO, GOLGA8J, EDRF1* and

ASF1A (Figure 3). In our case, the *TP53* mutation (p.G279E) occurred in the DNA binding domain and the *PTEN* mutation (exon5:c.492+1G>A) occurred in the splicing donor site of intron 5.

Discussion

The prognosis of primary nonseminomatous mGCTs in the absence of HMs is poor with a 5-year overall survival (OS) of 45%, compared with that of about 90% in pure seminoma irrespective of the primary site[1]. In comparison, the prognosis of patients with mGCT and associated HM is extremely poor, with a median OS of 5 months[5]. This dismal prognosis held true in the current case. The standard chemotherapy for GCT had little effect in this case. The induction therapy for AML did not improve the mGCT, and it grew larger. The AML-associated thrombocytopenia made it difficult to do the chemotherapy for the mGCT.

Previous research demonstrating isochromosome 12p in both GCTs and HMs suggested that these malignancies had a common progenitor, and the identification of the same gene mutations, including *TP53* and *PTEN,* in both mGCTs and AML samples in three cases, one of which is our case, established the idea that GCTs and AML share a founding clone[6, 9, 10]. In addition to *TP53* and *PTEN* mutations, we detected 7 commonly mutated genes in both tumors, even though their contributions to the tumor genesis have not been elucidated. In addition, 9 mutated genes were detected only in AML samples, while 7 mutated genes were detected only in GCT samples. These mutation profiles in AML and GCT strongly indicate that both originated from a common progenitor (Figure 3). The occurrence of 4 gene mutations as *PCLO*, *GOLGA8J, EDRF1,* and *ASF1A* on an initiator clone with *TP53, PTEN*, *RLF, DLG2,* and *YY2* mutations might have resulted in the establishment of the founder clone, which then developed separately along germ cell and hematopoietic lines by adding GCT- and AML-specific gene mutations, respectively. The progression of each tumor might have been mainly affected by its environment, and finally resulted in mGCT and AML, respectively. As mGCTs are cytogenetically identical to gonadal GCTs, they are thought to arise from the dissemination of early gonadal lesions[2]. The disseminated cells that recapitulate embryonal memory grow in the mediastinal region, and might develop into mGCTs. Hematopoietic cells traffic into and out of the thymus throughout postnatal and adult life via the thymic vasculature. The transforming cells with *TP53* and *PTEN* mutations in the mediastinal region might enter and do the homing in BM, just as occurs with lymphoid cells.

Two cases harboring concurrent mutations of *TP53* and *PTEN* in both mGCTs and AMKL have been reported, and our case is the third[9, 10]. The *TP53* mutation detected in our case (p.G279E) occurred in the DNA binding domain and might cause the impairment of TP53 function. The *PTEN* mutation (exon5:c.492+1G>A), in this case occurred in the splicing donor site of intron 5 resulting in a PTEN splicing mutant[12]. The same mutation has been reported in patents with Cowden syndrome, which causes hamartomatous neoplasms of the skin and mucosa, GI tract, CNS, and genitourinary tract, and an increased risk for malignancies of the breast, thyroid, endometrium[12]. *TP53* mutations have been widely observed in a variety of tumors, including AML, but they are uncommon in GCT[13]. Similarly, *PTEN* mutations have been widely reported in many types of tumors. In HMs, *PTEN* deletions and

mutations were detected in 10% and 27% of T-ALL cases, respectively, but the mutation in AML is rare[14]. Mice with heterozygous *PTEN* deletion demonstrated genomic instability and the development of multiple spontaneous tumors. The simultaneous depletion of TP53 and PTEN in mice promoted tumor genesis and metastasis[15], which might reflect the molecular pathology and the dismal prognosis of the concurrent disease of mGCT and AML.

Conclusions

Considering both the dismal prognosis and the characteristic mutation profiles revealed by WES analysis, the associated mediastinal GCT and AMKL harboring *TP53* and *PTEN* mutations should represent a unique biological entity.

Abbreviations

AFP: alpha-fetoprotein; AMKL: Acute megakaryoblastic leukemia; AML: Acute myeloid leukemia; BEP: Bleomycin, etoposide, and cisplatin; βhCG: beta-human chorionic gonadotropin; BM: bone marrow; CNS: Central nervous system; COSMIC: Catalogue of Somatic Mutations in Cancer; CT: Computed tomography; ESP: Exome Sequencing Project; GCT: Germ cell tumor; HM: Hematological malignancies; GI: Gastrointestinal; IGV: Integrative Genomics Viewer; mGCT: Mediastinal germ cell tumor; MDS: Myelodysplastic syndrome; OS: Overall survival; NCBI: National Center for Biotechnology Information; NHLBI: National Heart, Lung, and Blood Institute; SNP: Single nucleotide polymorphism; SNV: Single nucleotide variant; T-ALL: T-cell acute lymphobalastic leukemia; TGO: Paclitaxel, gemcitabine, oxaliplatin; TIP: Paclitaxel, ifosfamide, and cisplatin; VAF: variant allele frequencies; WES: Whole-exome sequencing

Declarations

Ethics approval and consent to participate

This study was approved by the Research Ethics Committee of the Faculty of Medicine, University of Miyazaki (G-0010) and we obtained written consent to participate in this study from the patient.

Consent for publication

We obtained written consent for publication about his clinical details and images from the patient and the patient's family.

Availability of data and materials

The datasets generated and/or analyzed during the current study are not publicly available due to individual privacy regulations but are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

Study conception and design: KS and KK. Acquisition of data: KA, YK, JK, MS, TK, KS, AK, YK, YT, TH, TK, and HT. Data analysis and interpretation: KA, TK, YK, KS, YS, HK, and KK. Writing and revision of the manuscript: KA, TK, YK, KK, and KS. Study supervision: KS and KK. All authors read and approved the final manuscript.

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Figures

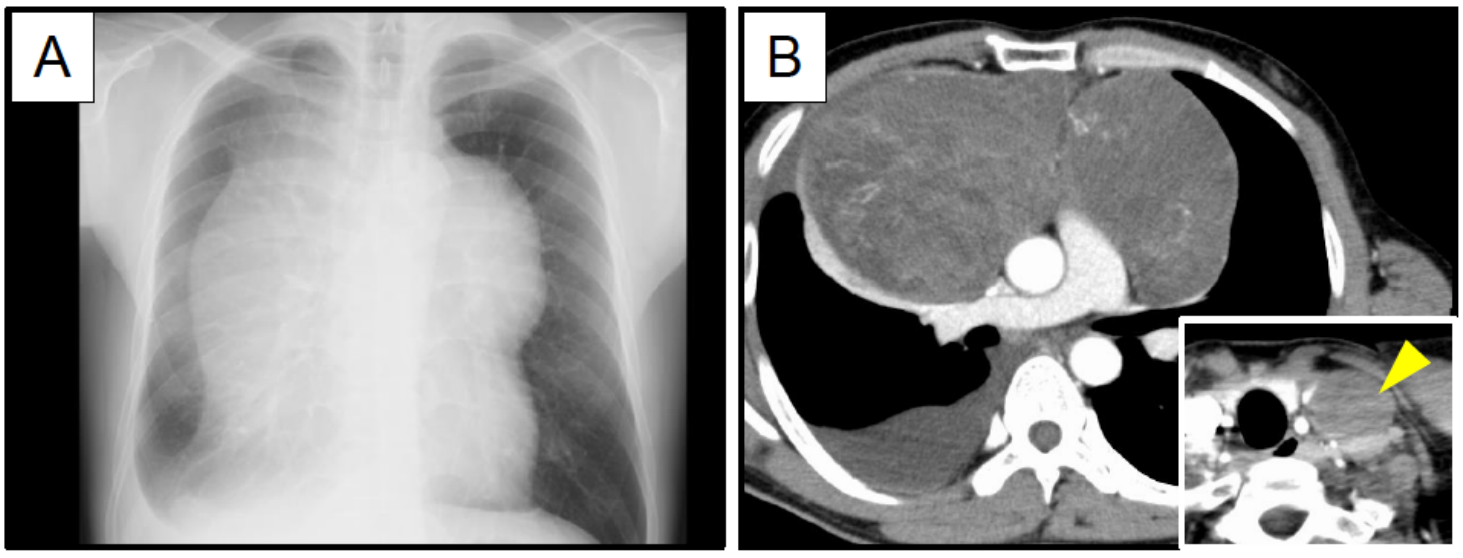

Figure 1

Chest X-ray and CT scan. (A) Chest X-ray reveals a well-circumscribed bilateral hilar mass approximately 20.5 cm in maximum dimension, and dullness of the right costal pleural angle. (B) CT scan reveals a 19.5

cm x 10.8 cm heterogeneously enhancing anterior mediastinal mass and a 4.3 cm left cervical mass (yellow arrow).

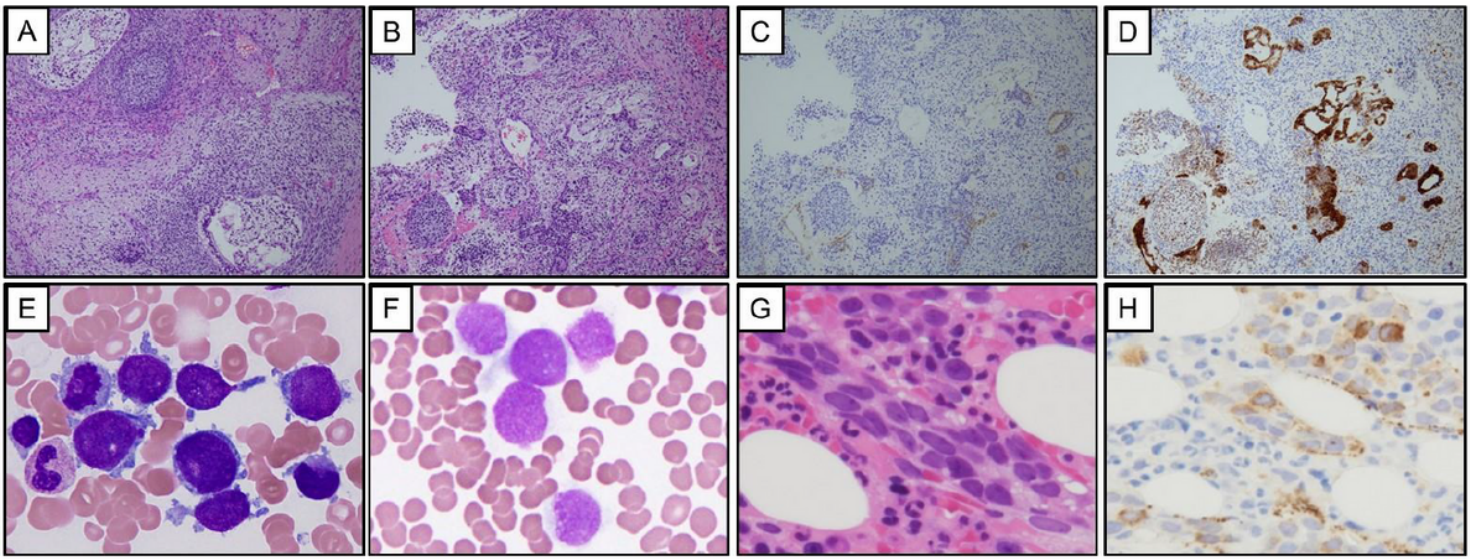

Figure 2

Histopathology of the left cervical mass shows features of non-seminomatous GCT. Cytology and histopathology of BM shows features of AMKL. (A) An open biopsy sample of the left cervical mass shows immature teratoma, columnar epithelium, and immature mesenchymal components (H.E., x200) and (B) yolk sac tumor-like components (H.E., x200). (C) Immunohistochemically, the yolk sac tumor-like components are weakly positive for AFP (x100) and (D) strongly positive for Glypican-3 (x100). (E) BM smear reveals many large blasts with nuclear and cytoplasmic blebs (Giemsa, x1000). (F) Blast cells are negative for myeloperoxidase. (G) BM biopsy shows increased blast cells (H.E., x400). (H) Immunohistochemically, blast cells show strong cytoplasmic positivity for von Willebrand factor (x400).

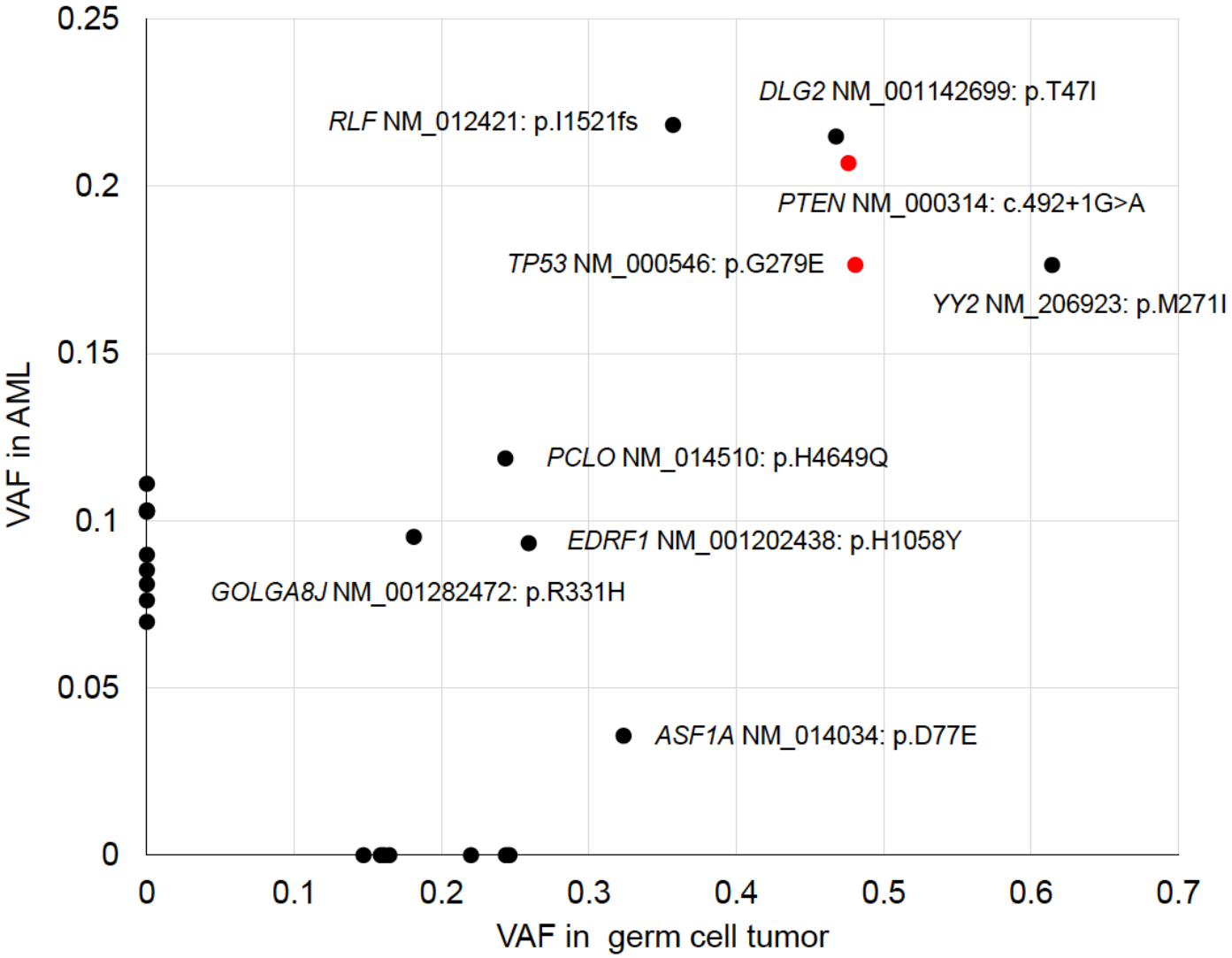

Figure 3

Mutated genes in GCT tumors and AML samples are plotted with their variant allele frequencies (VAFs). TP53 and PTEN mutations are colored red.