Inherited heterozygous Fanconi anemia gene mutations in a therapy-related CMML patient with a rare \textit{NUP98-HOXC11} fusion: A case report

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Fanconi anemia (FA) genes play critical roles in the repair of DNA lesions. Non-FA (or underlying FA) patients harboring heterozygous germline FA gene mutations may also face an increased risk of developing bone marrow failure, primary immunodeficiency disease, and hereditary cancer predisposition syndromes. We report a female patient who suffered from ovarian cancer at 50 years of age. During the initial treatment, six cycles of docetaxel and carboplatin (DC) combination chemotherapy were administered followed by two cycles of docetaxel maintenance therapy. Then, she received a routine follow-up every 3 months for the next 3 years, and all the results of the examination and laboratory tests were normal. Unfortunately, at 54 years of age, she developed a secondary cancer of therapy-related (t-) chronic myelomonocytic leukemia (t-CMML). After two courses of a highly intensive induction chemotherapy regimen with DAC (decitabine) and HAA (homoharringtonine, cytarabine), the patient suffered from severe and persistent bone marrow failure (BMF). Targeted next-generation sequencing (NGS) of a panel of 80 genes was performed on her initial bone marrow aspirate sample and identified \textit{PTPN11}, \textit{NRAS}, and \textit{DNMT3A} somatic mutations. In addition, RNA sequencing (RNA-seq) revealed a rare \textit{NUP98-HOXC11} fusion. Whole-exome sequencing (WES) verified \textit{RAD51C}, \textit{BRIP1}, \textit{PALB2}, and \textit{FANCG} heterozygous germline mutations of the FA pathway, which were further confirmed in buccal swab samples by Sanger sequencing. For this patient, we hypothesized that an altered FA pathway resulted in genomic instability, hypersensitivity to DNA-crosslinking agents or cytotoxic chemotherapeutics, and unsuccessful DNA damage repair. Consequently, she developed ovarian cancer and secondary t-CMML and then suffered from BMF and delayed post
chemotherapy bone marrow recovery after several chemotherapy courses. This case highlights the importance of genetic counseling in patients with hematopoietic neoplasms with high clinical suspicion for carrying cancer susceptibility gene mutations, which require timely diagnosis and personalized management.

**KEYWORDS**

Fanconi anemia gene, hereditary cancer predisposition syndromes, ovarian cancer, therapy-related CMML (t-CMML), NUP98-HOXC11 fusion

**Introduction**

The Fanconi anemia (FA) pathway (also known as the FA-BRCA pathway) is involved in the repair of DNA lesions by homologous recombination, which plays a vital role in the maintenance of genomic stability (1). To date, researchers have already identified germline mutations in 22 specific genes associated with the FA pathway, each accounting for an individual FA complementation group (2). Patients with FA gene mutation are hypersensitive to DNA damage and unable to successfully repair damaged DNA when exposed to DNA-crosslinking agents, cytotoxic chemotherapeutics, and ionizing radiation (3, 4). Throughout the lifetime of patients with an FA gene mutation, DNA damage increases accumulate, which would lead to a complex clinically and genetically heterogeneous disorder characterized by developmental abnormalities, bone marrow failure (BMF), immune deficiency, and a high risk of developing various cancers (e.g., Fanconi anemia, breast/ovarian cancer, leukemia) (5–7).

Here, we present the case of a 54-year-old female patient with multiple FA gene mutations. She was healthy, and with a normal history in her early years. Unfortunately, she developed ovarian cancer, secondary t-CMML, and post-chemotherapy BMF sequentially in her forties. Comprehensive genetic testing showed that many molecular variations (including FA gene germline mutations, RAS and epigenetic pathway somatic mutations, and NUP98-HOXC11 fusion) were highly linked to her serious and complex medical history (Figures 1A, B).

**Case report**

A 54-year-old female patient was admitted to the department of hematology in our hospital because of scattered ecchymosis on limb skin in July 2020. A complete blood count (CBC) test showed hyperleukocytosis and monocytosis with a white blood cell count (WBC) of 100.1×10^9/L, monocyte count of 47.1×10^9/L, hemoglobin level (HGB) of 72.0 g/L, and platelet count (PLT) of 16.0×10^9/L. An abdominal ultrasound scan showed that the thickness of the spleen was approximately 3.2 cm. Contrast-enhanced CT scans of the whole abdomen indicated no signs of ovarian cancer recurrence. Peripheral blood smear examination revealed monocytosis with an increased monocyte percentage of 56.0% and naïve monocyte percentage of 15.0% in total nucleated cells. Morphological examination of a bone marrow (BM) aspirate revealed dysplasia in granulocytic and megakaryocytic cell lineages and an increased naïve monocyte percentage (approximately 10%) (Figure 2A). BM biopsy analysis revealed marked hypercellularity (approximately 90%) with prominent naïve monocytes (Figure S1; Supplemental Material). Multiparametric flow cytometry analysis of BM aspirates showed that abnormal myeloid blasts with a percentage of 4.9% in total nucleated cells expressed the markers CD34, CD117, CD38, CD9dim, CD13dim, and CD33 and that naïve monocytes with a percentage of 8.4% in total nucleated cells expressed the markers CD64brig, CD11cbrig, CD15dim, and CD14neg (Figure S2; Supplemental Material). Cytogenetic analysis revealed an abnormal karyotype of “46, XX, t (11; 12)(p15; q13) [8]” (Figure 2B).

To clarify the molecular mechanism, comprehensive genetic testing was conducted (Supplemental Material). Targeted next-generation sequencing (NGS) of a panel of 80 genes was performed on her initial BM aspirate sample (Illumina NextSeq550 platform, amplicon library prep, amplicon mean coverage: 1813.4×, percent Q30 bases: 97.7%, uniformity of coverage: 97.8%) and identified PTPN11 (T73I: 2.5%), NRAS (G12D: 3.2%, G12S: 7.7%, G13V: 22.4%), and DNMT3A (A571fs*80: 49.1%) somatic mutations. In addition, RNA sequencing (RNA-seq) identified a rare NUP98-HOXC11 fusion. The breakpoints of NUP98 and HOXC11 were located in intron 12 or intron 1, respectively, resulting in a NUP98-HOXC11 in-frame fusion transcript containing exon 12 of NUP98 fused to exon 2 of HOXC11. To confirm the NUP98-HOXC11 fusion, reverse transcription polymerase chain reaction (RT-PCR) was performed with a pair of primers, forward (at NUP98 exon 12): 5’-TCTTGGTACAGGAGCCTTTGG-3’, and reverse (at HOXC11 exon 2): 5’-GTTCCCGGATCTGGAATTTCG-3’, and then the
amplified PCR products were purified to perform Sanger sequencing to verify the above finding (Figure 2C). Whole-exome sequencing (WES) of diagnostic BM samples identified four cancer susceptibility gene mutations associated with the FA-BRCA pathway, RAD51C (NM_058216.2: c.1027-1G>T), BRIP1 (NM_032043.2: c.2393G>A, p. R798Q), PALB2 (NM_024675.3: c.315G>C, p. E105D), and FANCG (NM_004629.1: c.1157C>G, p. P386R), which were further verified as heterozygous germline mutations by Sanger sequencing with buccal swab samples (Figure S3, Supplemental Material). RAD51C splicing mutation was predicted to be deleterious by the Human Splicing Finder and absent from the current Exome Aggregation Consortium (ExAC), 1000 Genomes Project (1000G), and Genome Aggregation Database (gnomAD), indicating that it may be a pathogenic/likely pathogenic variant. BRIP1, PALB2, and FANCG missense mutations were all rare in the current ExAC, 1000G, and gnomAD databases (Minor allele frequencies < 0.0001), and reported in ClinVar under “uncertain significance.” Furthermore, no other exonic variants were detected in genes associated with predisposition to cancer.

The patient’s past medical history provided the medical context that she had developed ovarian cancer with no history of cancer in her family in September 2016 (four years prior). Then she received six cycles of docetaxel and carboplatin (DC) combination chemotherapy [docetaxel, intravenous (I.V.) infusion by 60 minutes, 70 mg/m², d1; carboplatin, I.V. infusion over 30 minutes, AUC (area under the plasma-concentration-versus-time curve) of 5 mg/mL/min, d1], followed by two cycles of docetaxel maintenance therapy (docetaxel, I.V. infusion by 60 minutes, 70 mg/m², d1) after a definitive diagnosis from October 2016 to May 2017 in the medical oncology department of our hospital. After the primary
After receiving the diagnosis, she underwent a routine follow-up nearly every 3 months for the next 3 years. All the obtained results of the examination and laboratory tests were normal, and she had no signs or symptoms of ovarian cancer recurrence.

Based on the above results, the patient’s final diagnosis was t-CMML with the NUP98-HOXC11 fusion and secondary to primary ovarian cancer with inherited cancer predisposition gene mutations of FA genes. Considering the extremely poor prognosis, two courses of a highly intensive induction chemotherapy regimen with DAC (decitabine, 20 mg/m², d1-5) and HAA (homoharringtonine, 2 mg/m², d1-7; cytarabine, 10 mg/m², q12h, d1-10) were administered from 2 July 2020 to 20 August 2020. Subsequently, the patient presented with obvious pancytopenia and suffered from severe and persistent bone marrow suppression and delayed post-chemotherapy bone marrow recovery with a poor response to G-CSF treatment. On 2 September 2020, a CBC test indicated remaining pancytopenia with a WBC of 0.9×10⁹/L, HGB of 73.0 g/L, and PLT of 36.0×10⁹/L. Then, the patient asked to be discharged and transferred to her local hospital for personal reasons.

**Discussion**

Fanconi anemia (FA) is a rare human genetic disease that occurs following germline mutations associated with the FA pathway and is clinically characterized by malformations, BMF, immune deficiency, and an extremely high predisposition to various cancers. The “FA pathway” is also known as the “FA-BRCA pathway” because many of the FA genes are related to breast cancer (BRCA) and/or ovarian cancer (7, 8). FA patients display a wide spectrum of diverse clinical manifestations; even so, only a small number of them present a typical phenotype, and one-third of individuals with FA have a normal appearance (6). Thus, it is a major challenge for clinicians to make an accurate diagnosis for FA.
patients. Chromosome breakage analysis may be a gold standard test, which is, nonetheless, not widely applied due to its false-negative traits and the inaccessibility in some poorly equipped hospitals (9, 10). According to previous literature, non-FA (or underlying FA) patients carrying germline FA gene mutations also present with an increased risk of developing BMF, immune deficiency, and cancer susceptibility under some circumstances (5, 11, 12).

Our patient had no signs or symptoms of developmental abnormalities or organ defects, and unfortunately, she did not receive a chromosome breakage test. Genetic testing indicated that she was born with four FA-BRCA pathway gene mutations in RAD51C, BRIP1, PALB2, and FANCG. Moreover, RAD51C (RAD51 paralog C, alias FANCO), BRIP1 (BRCA1-interacting protein carboxy-terminal helicase 1, alias FANCJ), and PALB2 (partner and localizer of BRCA2, alias FANCN) have been fully implicated in an increased risk for breast and ovarian cancer (Table 1) (13–15). We hypothesize that the patient was possibly born with an increased predisposition to develop BMF, hematopoietic neoplasms, and breast and ovarian cancer due to a mild or moderate impaired FA-BRCA pathway.

At the cellular level, her cells are hypersensitive to DNA-crosslinking agents, cytotoxic chemotherapeutics, and ionizing radiation due to corrupted DNA damage repair (3, 5). Over time, cumulative genomic instability renders cells malignant by clonal evolution and neoplastic transformation through uncontrolled proliferation, escape from programmed cell death, and evasion of immune surveillance. Consequently, she developed ovarian cancer at 50 years of age and then received six cycles of docetaxel and carboplatin (DC) combination chemotherapy followed by two cycles of docetaxel maintenance therapy. Cytotoxic chemotherapeutic exposure aggravated the tendency of hematopoietic stem progenitor cells to undergo malignant transformation and BMF. Three years later, she suffered from a secondary cancer of t-CMML and severe and persistent BMF after two courses of an induction chemotherapy regimen with DAC-PTPN11 fusion (23, 24). Since then, it has been identified in a spectrum of hematologic malignancies, including AML, MDS, CML, T-ALL, and mixed-phenotype acute leukemia (MPAL), leukemogenesis through complex molecular mechanisms (18). NUP98 fusion is rarely reported in CMML fusion cases reported by Taketani T et al. and NUP98-HOXC11 fusion cases were first described by Nakamura T et al. and first performed in a pediatric patient and three in adult patients with de

**TABLE 1** Association of defects in FA genes with predisposition to non-FA cancers.

| References     | Gene Pathway             | Function                             | Hematologic malignancy | Other malignancy            |
|----------------|--------------------------|--------------------------------------|------------------------|-----------------------------|
| Meindl A et al. (13) | RAD51C/ FANCO Fanconi anemia/BRCA pathway | Regulate DNA repair by homologous recombination, and maintain genome stability | MDS, leukemia           | Breast-ovarian cancer       |
| Moyer CL et al. (14) | BRIP1/FANCI Fanconi anemia/BRCA pathway | Regulate DNA repair by homologous recombination, and maintain genome stability | MDS, leukemia           | Ovarian cancer, Breast cancer |
| Antoniou AC et al. (15) | PALB2/FANCN            |                                      | MDS, leukemia           | Breast cancer               |
| Nepal M et al. (5)    | FANCG                   |                                      | MDS, leukemia           |                             |

FA, Fanconi anemia; MDS, Myelodysplastic Syndromes.
novo AML. To the best of our knowledge, this is the first report identifying a rare NUP98-HOXC11 fusion in a t-CMML patient.

In summary, we report the first case of a non-FA (or underlying FA) patient born with multiple FA gene mutations who developed ovarian cancer and secondary t-CMML with a rare NUP98-HOXC11 fusion. Conventional chemotherapy and radiation therapy appear to be “double-edged swords”, as they kill cancer cells and induce secondary BMF, immune deficiency, and cancer (32). Particularly, it is difficult for clinicians to balance the clinical efficacy and adverse outcomes in cancer patients carrying cancer susceptibility gene mutations. From this case, we learn that cancer patients with high clinical suspicion for germline predisposition, such as secondary or early-onset hematologic malignancy patients, are desirable for appropriate genetic counseling and testing. Actually, those patients may benefit from timely diagnosis and personalized management, such as a better donor selection strategy, reduced-intensity conditioning for allogeneic hematopoietic stem cell transplantation, altered cytotoxic chemotherapeutics, and lifelong cancer surveillance.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: https://www.ncbi.nlm.nih.gov/bioproject/PRJNA881251/.

Ethics statement

The studies involving human participants were reviewed and approved by The Medical Ethics Committee of Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology. The patients/participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

Author contributions

KS collected and analyzed data, wrote the manuscript, and drew the figures. MZ performed RNAseq and analyzed data. JCW performed NGS and analyzed data. WM performed WES and analyzed data. JW performed morphological examination. CW performed flow cytometry and analyzed data. SX performed cytogenetic analysis. ZH and MX directed the research and reviewed the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2022.1036511/full#supplementary-material

| References       | Partners | Disease | Age | Sex | Karyotype | Primary cancer     | Latency | Exposure                              |
|------------------|----------|---------|-----|-----|-----------|--------------------|---------|---------------------------------------|
| Hatano et al. (26) | HOXA9    | CMML    | 45  | M   | 46, XY; t(7;11)(p15;p15) | .       | .                                    |         |
| Wong et al. (27)  | HOXA9    | CMML    | 31  | M   | 46, XY, t(7;11)(p15;p15) | .       | .                                    |         |
| Soler G et al. (28) | AF10    | CMML    | 83  | M   | 46, XY, t(10;11)(p12;p15) | .       | .                                    |         |
| Hayashi Y et al. (29) | HBO1    | t-CMML  | 69  | F   | 46, XX, t(11;17)(p11.5;q21) | Gastric cancer | 3 year | fluorouracil,cisplatin,docetaxel,gemcitabine |

CMML, chronic myelomonocytic leukemia; t-CMML, therapy-related chronic myelomonocytic leukemia; M, male; F, female.
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