MDS/MPN-Unclassifiable with $t(X;17)(q28;q21)$ and *KANSL1-MTCP1/CMC4* Fusion Gene

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Established Facts

- MDS/MPN-unclassifiable is a heterogeneous category of diseases.
- Underlying genetic alterations in MDS/MPN-unclassifiable are not well characterized.

Novel Insights

- This is the first case of MDS/MPN with a $t(X;17)$ translocation leading to *KANSL1-MTCP1* and *KANSL1-CMC4* fusion genes.
- As in T-PLL, the overexpression of oncogenic protein MTCP1 might have a pathogenic role in myeloid neoplasms.

Keywords

Myelodysplastic/myeloproliferative disorders · *KANSL1-MTCP1* fusion · *KANSL1-CMC4* fusion

we report a case of MDS/MPN-U with a $t(X;17)(q28;q21)$ chromosomal rearrangement leading to the *KANSL1-MTCP1* fusion gene.

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Abstract

Myelodysplastic/myeloproliferative neoplasm, unclassifiable (MDS/MPN-U) is a poorly characterized entity among overlap myeloid syndromes. Recent studies have shown heterogeneous mutational profiles in this group being able to subclassify them into entities closely related to the more well-established disorders under the umbrella term of the MDS/MPN group. Recurrent cytogenetic alterations are, nonetheless, rare in MDS/MPN-U. Here, for the first time,

Introduction

Myelodysplastic/myeloproliferative neoplasm, unclassifiable (MDS/MPN-U) has morphological features that overlap with both myelodysplastic syndrome (MDS) and myeloproliferative neoplasm (MPN) categories but

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do not meet the criteria for any specific MDS/MPN [Swerdlow et al., 2017]. It is the most poorly established entity among all subtypes of MDS/MPN overlap syndromes [Arber et al., 2016]. There are no specific molecular genetic findings in MDS/MPN-U. Several studies have described *ASXL1*, *TET2*, *JAK2*, *SETBP1*, *NRAS*, *EZH2*, *RUNX1*, and *CBL* mutations in different frequencies [Meggendorfer et al., 2013; Wang et al., 2014; Zoi and Cross, 2015; Bose et al., 2018]. The majority of MDS/MPN syndromes show a normal karyotype, however, recurrent cytogenetic changes are seen more frequently in the MDS/MPN-U when compared to the other MDS/MPN entities, with trisomy 8 and del(7q) being the most common changes identified [Bose et al., 2018]. Recently, a novel t(X;17)(q28;q21) chromosomal rearrangement was described in a case of acute monocytic leukemia resulting in *KANSL1-MTCP1* and *KANSL1-CMC4* fusion genes [Li et al., 2020]. Herein, we describe, to the best of our knowledge, the first case of MDS/MPN-U with a t(X;17)(q28;q21) chromosomal rearrangement.

Case Presentation

An asymptomatic 42-year-old man was found to have an abnormal complete blood count during his annual family physician visit. Blood tests indicated hemoglobin of 115 g/L, leukocytes of $34 \times 10^9/L$, platelet count of $400 \times 10^9/L$, absolute neutrophil count of $22.7 \times 10^9/L$, and absolute monocyte count of $5.7 \times 10^9/L$. Rare circulating blast cells were present. CT scan showed a mildly enlarged spleen of 15 cm but no lymphadenopathy or hepatomegaly. Bone marrow evaluation showed MDS/MPN-U with 12% blast cells of myeloid phenotype expressing CD13, CD33, CD34, CD117, and HLA-DR but not CD11b, CD14, CD35, and CD300e. Trilineage dysplasia was observed with a mild increase in mature monocytes. The G-banded karyotype showed a balanced translocation involving chromosomes X and 17 in 16 of 20 metaphases (Fig. 1a). The breakpoints of Xq28 and 17q21 are not recognized as breakpoints involved in a frequent recurrent rearrangement in myeloid neoplasia. However, a literature search suggested a rare t(X;17) that fuses the *KANSL1* gene with both *MTCP1* and *CMC4* genes in a case of acute myeloid leukemia [Li et al., 2020]. The *MTCP1* and *CMC4* genes overlap and share several exons. Since commercial fluorescence in situ hybridization (FISH) probes were unavailable, custom-labeled BACs were ordered (Centre for Applied Genomics, Toronto, Canada). The *MTCP1* locus on Xq28 (orange label) was represented by BAC probe RP11-402H20, and the *KANSL1* locus on 17q21 (green label) was represented by RP11-904E20 and RP11-669E14. FISH was performed on metaphases that had previously been analyzed by karyotyping and shown to carry the t(X;17). Sequential G-banding-to-FISH showed a co-localization of the *MTCP1* and *KANSL1* probes on both derivative chromosomes, der(X) and der(17), indicating a rearrangement involving these gene regions (Fig. 1b). Seven abnormal metaphases were analyzed that showed fusion of the *KANSL1* and *MTCP1/CMC4* probes. The remaining normal

metaphase did not show a fusion of the *KANSL1* or *MTCP1/CMC4* probes. An interphase FISH analysis was not performed.

Further, we performed Optical Genome Mapping (OGM, Bionano Genomic Saphyr), showing a balanced translocation between chromosomes X and 17 (Fig. 1c). Since OGM has a higher resolution than either karyotyping or FISH, it demonstrated that the translocation breakpoints are, in fact, within the *KANSL1* and *MTCP1/CMC4* genes (Fig. 1d). RT-PCR was also performed to confirm the presence of a chimeric fusion protein between *KANSL1* and *MTCP1/CMC4* (Fig. 1e).

Next-generation sequencing, examining DNA sequence variants in the exonic coding regions of 49 clinically relevant genes in hematological malignancies, showed the presence of the *JAK2* V617F (variant allele frequency [VAF], 16%), *SETBP1* (VAF, 27%), *U2AF1* (VAF, 41%), and 2 variants of uncertain significance in *CTNNA1* (VAF, 51%) and *TP53* (VAF, 52%). The *TP53* variant (p.Arg290Cys) had been previously reported in hematological malignancies and affected the DNA-binding domain of the gene. However, functional studies have shown that this variant preserves transactivation activity equivalent to the normal p53 protein and is not expected to disrupt the transcriptional activity of the encoded protein (IARC *TP53* database; p53.iarc.fr); it was, therefore, classified as a variant of uncertain significance [Petitjean et al., 2007].

The therapeutic plan for this patient was a fully matched allogeneic bone marrow transplant, so he was started on azacytidine to control the disease. His sister was found to be HLA identical.

Fig. 1. a G-banded karyogram illustrating the t(X;17)(q28;q21). Red arrows indicate the breakpoints of the rearrangement on chromosomes X and 17. **b** Sequential G-banding-to-FISH (inverted DAPI) performed on previously karyotyped metaphases shows the co-localization of the RP11-402H20 (*MTCP1/CMC4* region, orange) and RP11-904E20/RP11-669E14 probes (*KANSL1* region, green) on the derivative X and derivative 17 chromosomes indicating a translocation (red and green probe fusion signals). The normal chromosome 17 shows only the green signal of the *KANSL1* probe. **c** Optical genome mapping (OGM) circo plot also showing a reciprocal translocation between chromosomes X and 17 represented by the pink line in the centre of the plot (red arrow). **d** OGM detailed genome view shows a hybrid molecule (blue bar) aligning to the chromosome 17 reference (green bar, top) and the chromosome X reference (green bar, bottom). The breakpoint is estimated in the exon 1–2 region of *KANSL1* and in the 5' region of the *MTCP1* and *CMC4* genes. Direction of transcription of each gene is indicated by a text arrow beside the gene name. **e** RT-PCR using published primers [Li et al., 2020] for the *KANSL1-MTCP1* and *KANSL1-CMC4* fusions. PCR in the left lane (no template control) does not produce a product, neither do the primers with control RNA (second lane). The K4F, M3R primers produce a faint 233-bp band (third lane), and the K4F, C4R primers show a strong fusion product at 366 bp (fourth lane) at an annealing temperature of 59°C. PCR primers and conditions were performed as outlined in Li et al. [2020]. K4F - GGGCTACGCTCCTCCATCA (*KANSL1* NM_001193465.1), M3R - CACATCCTCTCCTGCCATTCT (*MTCP1* NM_001018025.4), and C4R - ACTGAGCACAACT-TACG (*CMC4* NM_001018024.3).

(For figure see next page.)

While the transplant was delayed due to COVID pandemic, several follow-up bone marrow aspirates and biopsies were performed, all consistent with persistent disease showing between 6 and 13% blasts and moderate control of peripheral blood counts.

Of note, the patient never had a sustained absolute peripheral monocytosis. He ultimately underwent a fully matched allogeneic bone marrow transplant from his sibling. His most recent bone marrow (day +60) did not show any evidence of disease.

Discussion

MDS/MPN-U is associated with a median overall survival of approximately 12–21 months [DiNardo et al., 2014; Wang et al., 2014]. Of all the MDS/MPNs, the genomic profile of MDS/MPN-U is the least well characterized, which is unsurprising given that its diagnosis is predicated on the failure to meet criteria for any other specific MDS/MPN. Recent studies, however, have shed some more light on this area [Wan and Han, 2020]. Palomo et al. [2020] performed mutational analyses on the largest well-annotated MDS/MPN cohort to date, a total of 367 patients, of which 106 had MDS/MPN-U. The combination of 2 frequently mutated genes, *SETBP1* (as an ancestral mutation) and *JAK2* (as a secondary mutation), is most commonly seen in MDS/MPN-U, among other subgroups [Palomo et al., 2020]. They utilized molecular signatures identified in the other MDS/MPNs to subclassify these cases as CMML-like, aCML-like, MDS/MPN-RS-T-like, as well as a *TP53* mutated and an “Other” group. Our case showed mutations in *JAK2* V617F, *U2AF1*, *TP53*, and *SETBP1*. However, the mutational profile of our case does not match any of their more well-characterized groups, although the presence of *U2AF1* and *JAK2* in conjunction with thrombocytosis was more in keeping with the “Other” group [Palomo et al., 2020].

To our knowledge, this is the first case to show a t(X;17)(q28;q21) resulting in *KANSL1-MTCP1* and *KANSL1-CMC4* fusion genes in an MDS/MPN. Little is published in the medical literature concerning *MTCP1/CMC4* and myeloid neoplasms. A recent paper by Li et al. [2020] has helped characterize the role of the t(X;17)(q28;q21) *KANSL1-MTCP1* gene fusion in a case of acute myeloid leukemia, and more specifically, elucidated the function of *MTCP1* in myeloid neoplasia. *MTCP1* is not expressed under physiological conditions, but aberrant expression causes increased cell proliferation and a partial block in differentiation. A t(X;17)(q28;q21) has also been reported as the sole karyotypic anomaly in what was described as a morphological subtype of acute promyelocytic leukemia, which did not demonstrate a PML-RAR α rearrangement by FISH, suggesting, in our opinion, that *MTCP1* (located in Xq28) may have played a role in its pathogenesis although this is somewhat speculative [Wang et al., 2010].

MTCP1 has been studied more extensively in the context of T-cell prolymphocytic leukemia (T-PLL) [Madani et al., 1996; Gritti et al., 1998; De Schouwer et al., 2000]. In this context, *MTCP1* has been shown to be a 3D structural analog of *TCL1* [Petock et al., 2002]. The mecha-

nism involved in T-PLL, however, involves placing *MTCP1* under the control of regulatory sequences of a T-cell receptor gene resulting in increased *MTCP1* expression, as opposed to forming a fusion protein. Members of the *TCL1* proto-oncogene family (*TCL1*, *MTCP1*, and *TCL1b*) are known to bind to Akt1, increasing its phosphorylation status and kinase activity and allowing enhanced signal transduction, cell proliferation, and survival [Laine et al., 2002; Sun and Fang, 2020]. While not completely elucidated, it appears that the presence of *TCL1* translocation or protein expression is associated with poor outcomes in T-PLL [Jain et al., 2017]. If the presence of an *MTCP1* translocation portends a better prognosis is not fully understood.

MDS/MPN-U remains a poorly defined MDS/MPN subcategory in the WHO classification of hematopoietic tumors despite some recent progress in our understanding of its molecular landscape [Palomo et al., 2020; Wan and Han, 2020]. Lack of *ASXL1* and presence of *JAK2* are shown to be associated with better prognosis in MDS/MPN overlap syndromes [Cannella et al., 2008; Patnaik et al., 2014; Cui et al., 2015]. The effect of *MTCP1* expression in the prognosis of myeloid neoplasm is not known. Our patient was in complete remission at the latest follow-up, about 1 year from diagnosis. Our discovery of a t(X;17)(q28;q21) *KANSL1-MTCP1/CMC4* gene fusion, a novel finding in the case of MDS/MPN-U, further enhances our knowledge of the underlying potential genetic drivers of this diverse entity as well as provides an additional example of this extremely rare gene fusion in the context of myeloid neoplasia.

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Statement of Ethics

As a case report, the study was granted an exemption from requiring ethics approval from the “Research Ethics Board” of the University Health Network. The study was conducted in compliance with the Declaration of Helsinki. Informed written consent was obtained from the patient to publish the details of medical conditions and treatment regimens.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

A.S.: study design, acquisition, assembly, analysis and interpretation of data, drafting of the manuscript, critical revision of the manuscript for important intellectual content. P.M.: study design, analysis, and interpretation of data, drafting of the manuscript. A.C.S.: study design, acquisition and interpretation of cy-

togenetic data, drafting of the manuscript, critical revision of the manuscript for important intellectual content. S.S.: acquisition, analysis, and interpretation of molecular data, drafting of the manuscript, critical revision of the manuscript for important intellectual content.

Data Availability Statement

All data generated or analyzed during this study are included in this article. Further inquiries can be directed to the corresponding author.

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