Received: 17 July 2023 | Revised: 25 August 2023 | Accepted: 9 September 2023

DOI: 10.1002/ajh.27108

CORRESPONDENCE

CMSS1::FLT1 rearrangement leads to ligand-independent activation of FLT1 signaling in acute myeloid leukemia

To the Editor:

The human genome contains 90 genes that encode either receptor or non-receptor tyrosine kinases, most of which are historically oncogenes activated by mutations, amplifications, in-frame indels, or rearrangements.[1] Rearrangement of tyrosine kinases, which are often fused with a dimerization domain-containing fusion partner, is a potent mechanism for constitutive kinase activation that causes uncontrolled cell proliferation and is found in a variety of tumors. However, the rearrangement of the vascular endothelial growth factor receptor (VEGFR) family has never been reported in cancer, which may indicate that the obstruction of neoangiogenesis, which is critical for tumor development and requires VEGFRs' involvement, poses a significant obstacle for tumor cells to overcome. Here, we describe a de novo acute myeloid leukemia (AML) case in which a translocation between chromosome 3q12 and chromosome 13q12 leads to a gene fusion between *FLT1* (also known as *VEGFR1*) and *CMSS1* (Cms1 ribosomal small subunit homolog). The resulting chimeric protein contains an N-terminal fragment from CMSS1 and the intact tyrosine kinase domain of FLT1. CMSS1::FLT1 is cytoplasmic, constitutively activated, and promotes cell proliferation. In vitro cell models with forced expression of CMSS1::FLT1 were sensitive to an FLT1 kinase inhibitor Axitinib.

A 79-year-old female patient presented with decreased WBC. Physical examination revealed no bruising or organomegaly. The complete blood counts showed WBC 1.8×10^9/L, Hb 105 g/L, MCV 109.2 fL, PLT 213×10^9/L, 3% blasts, 38.6% neutrophils, 47.5% lymphocytes, 13.3% monocytes, 0.6% eosinophils, and 0% basophils. Bone marrow aspirate smear revealed 21% of variably sized blasts with cytoplasmic pseudopod formation and 1–3 nucleoli. The myeloid elements were decreased and exhibited left-shift maturation, with some cells containing increased cytoplasmic granules. Erythroblasts were significantly increased, with some binucleated erythroblasts. Occasional erythrocytes with Howell–Jolly bodies were observed (Figure 1A). The myeloid to erythroid ratio was 0.35:1. Cytochemical analysis of blast cells was negative for myeloperoxidase (MPO) and periodic acid–Schiff (PAS). Bone marrow flow cytometry revealed 28% of CD34+ cells that were CD13+, CD33+, CD34+, CD38dim, CD117+, CD200+, HLADRdim, CD2−, cyCD3−, CD5−, CD7−, CD11b−, CD14−, CD16−, CD19−, CD56−, cyCD79−, and MPO−. The diagnosis of AML was made. Cytogenetic analysis showed a complex rearrangement between chromosome 3 and 13 as the sole change (46,XX,der(3)t(3;13)(q12;q12)inv(3)(p25q11.2),der(13)t(3;13)[10]/46,XX[10]) (Figure 1B). A targeted DNA-NGS assay of 128 leukemia-related genes identified *BCORL1* p.A427Dfs17 (VAF 10.6%) and *ETV6* p.R103Sfs9 (10.4%). CNV analysis revealed no apparent chromosome gain or loss. A targeted RNA-NGS assay with bait probes covering 81 leukemia-related genes did not find any fusion transcript. After obtaining the patient's informed consent and approval from our local Institutional Review Board (IRB), we performed a genome-wide mate-pair DNA sequencing, which revealed DNA fusion fragments consistent with the chromosome breakpoints seen by karyotype analysis, containing intron 1 of *CMSS1* at 3q12 and intron 15 of *FLT1* at 13q12 (Figure 1C). The expression of the *CMSS1*::*FLT1* fusion transcript was confirmed by a RT-PCR assay with primers specific to *CMSS1* (*CMSS1*_F1: TACCCGTGATGTTCTGC; *CMSS1*_F2 nest: TCGAGACCTGAGCTGAAA) and *FLT1* (*FLT1*_R1: ACTTGCTGGCATCATAAG; *FLT1*_R2 nest: GAAGACCTTTTCATTTTTCGG), which showed two distinct bands in an agarose gel electrophoresis (Figure 1D). Sanger sequencing of the major band showed an in-frame fusion between *CMSS1* exon 1 and *FLT1* exon 16. The predicted chimeric protein contains an N-terminal fragment from CMSS1 and the entire tyrosine kinase domain from FLT1. The minor band consisted of *CMSS1* exon 1, a 50 bp *CMSS1* intronic sequence from intron 1, and *FLT1* exon 16 (Figure 1E), which is likely from differential splicing because a classic acceptor splicing signal "AG" and a donor splicing signaling "GT" were observed at the 5′ and 3′ end of the inserted intronic fragment, respectively. The minor transcript is likely nonfunctional due to a shifted reading frame.

A lentivirus carrying an *MYC*-tagged *CMSS1*::*FLT1* was introduced into NIH3T3 cells. The subcellular localization of CMSS1::FLT1 was determined using immunofluorescence staining with an anti-MYC antibody, which revealed predominant cytoplasmic localization (Figure 1F). An immunoprecipitation of CMSS1::FLT1 was performed with MYC antibody, which showed a band corresponding to the expected size of CMSS1::FLT1 (72 Kd). Upon stripping and incubating with an anti-phospho-tyrosine antibody, the CMSS1::FLT1 was tyrosine phosphorylated (Figure 1G). In addition, MAPK, a known downstream target of FLT1 activation, is also phosphorylated (Figure 1H). These results are consistent with a ligand-independent kinase activation of the CMSS1::FLT1. CMSS1::FLT1-expressing cells showed significantly faster growth compared to cells expressing an empty vector, as determined by the Cell Counting Kit-8 (CCK-8) assay (Figure 1I). In contrast, cells expressing CMSS1::FLT1 were significantly more sensitive to treatment with Axitinib, an FLT1/VEGFR1 kinase inhibitor (Figure 1J).

The VEGFR family comprises three members: FLT1 or VEGFR1, kinase insert domain receptor (KDR) or VEGFR2, and FLT4 or VEGFR3. These tyrosine kinase receptors possess seven immunoglobulin (Ig)-like extracellular domains, a transmembrane domain, and an intracellular split

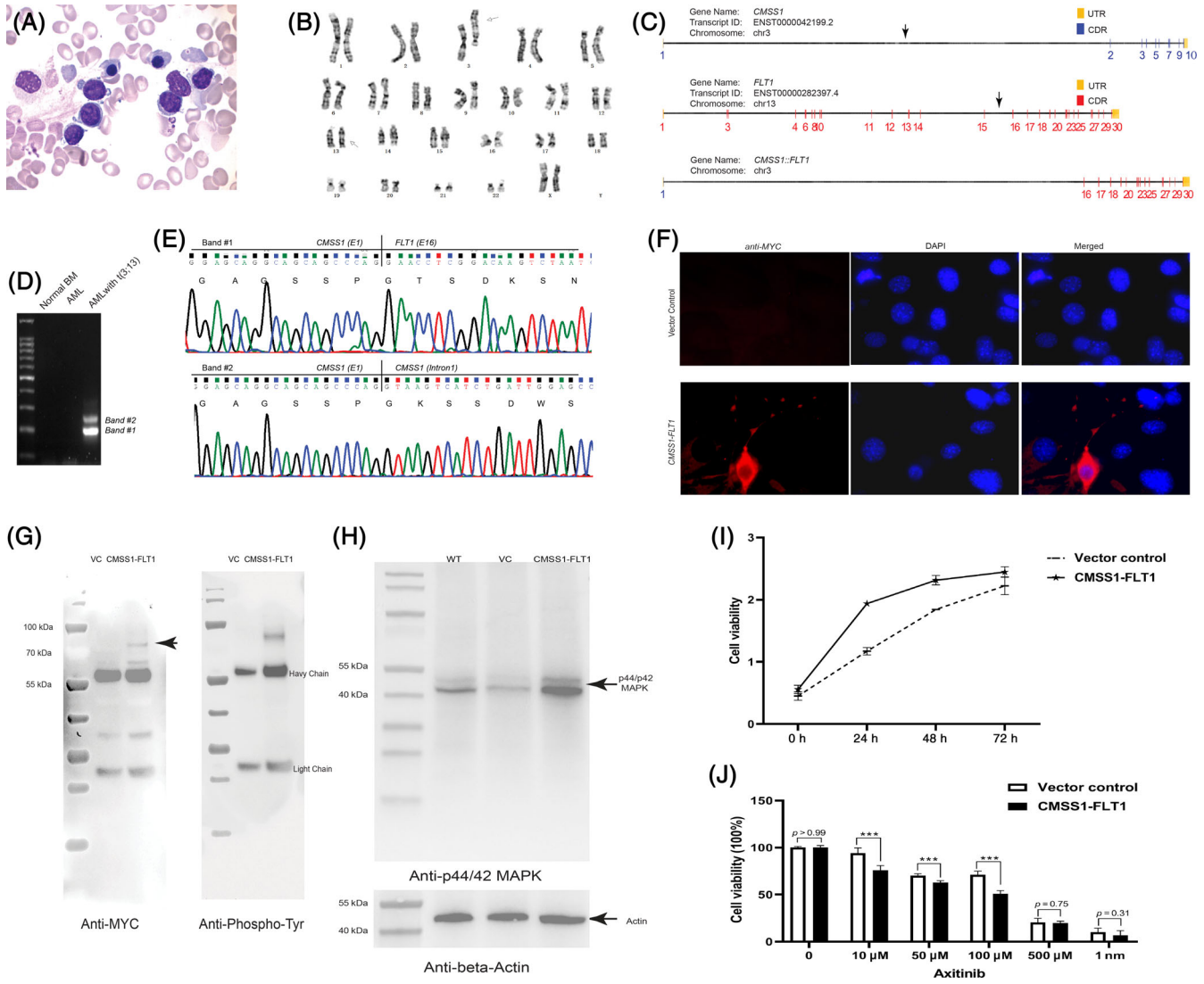

FIGURE 1 (A) Bone marrow smear showed variably sized blasts with cytoplasmic pseudopod formation; (B) Karyotype analysis of bone marrow aspirates showed a rearrangement between chromosomes 3 and 13 (arrows) as the sole change; (C) Genomic structure of *CMSS1*, *FLT1*, and the *CMSS1*::*FLT1* fusion gene. Breakpoints of *CMSS1* and *FLT1* are marked with arrows. (D) RT-PCR with primers specific to *CMSS1* and *FLT1* detected 2 *CMSS1*::*FLT1* fusion transcripts in the patient's specimen but not in the nonneoplastic bone marrow or an AML without the (3;13). (E) Sanger sequencing of the RT-PCR products confirmed an in-frame fusion between exon 1 of *CMSS1* and exon 16 of *FLT1* (band #1). The minor transcript (band #2) is a product of differential splicing, which is frameshifted and presumably nonfunctional. (F) The CMSS1::FLT1 fusion was cytoplasmic in NIT3T3 cells by immunofluorescence stain; (G) The CMSS1::FLT1 fusion in NIH 3T3 cells was pulled down by immunoprecipitation and stained with anti-MYC (left panel) and anti-phosphotyrosine antibody (right). These results suggest ligand-independent kinase phosphorylation of the CMSS1::FLT1 (arrow); (H) A Western blot showed increased phosphorylation of MAPK in cells expressing CMSS1::FLT1 compared to cells expressing the vector control; (I) Cell proliferation was significantly increased in CMSS1::FLT1-expressing cells compared to vector control cells by the CCK8 assay at different time points; (J) CMSS1::FLT1-expressing cells were more sensitive to Axitinib at lower doses compared to vector control cells. ***p <0.001 (Student's t-test). AML, acute myeloid leukemia; BM, bone marrow; CDR, coding region; UTR, untranslated region; Vc, vector control; WT, wild type.

tyrosine kinase domain. Both FLT1 and KDR are predominantly expressed in vascular endothelial cells, while FLT1 is also expressed in monocytes and macrophages.[2] FLT4 is mainly expressed in lymphatic endothelial cells. During embryonic development, FLT1 and KDR are required for angiogenesis and vasculogenesis, with KDR being the main signaling transducer and FLT1 a major regulator of VEGFA (ligand) levels. FLT4 plays a crucial role in lymphatic vessel development. Recent studies indicate that all three VEGFRs are important for neoangiogenesis in tumors. VEGFR amplification, activation mutation, and overexpression have been observed in various tumors, including lung adenocarcinoma, colon adenocarcinoma, melanoma, glioma, and endometrial endometrioid adenocarcinoma.[3] FLT1 has been implicated in tumor metastasis by promoting the recruitment and activation of macrophages in the tumor microenvironment. FLT1 activation on macrophages can result in the secretion of cytokines and growth factors that promote tumor growth and invasion, as well as the recruitment of additional macrophages to the tumor site. FLT1 signaling on tumor cells can also contribute to metastasis by promoting the development of a pre-metastatic niche and enhancing tumor cell

migration and invasion. In a mouse model expressing a deficient FLT1 with its tyrosine kinase domain deleted, highly metastatic 3LL-LLC lung cancer cells failed to produce lung metastasis. Macrophages with a tyrosine kinase-deficient FLT1 lost the capability of cell migration when induced by VEGF.[4]

While FLT1 is well known for its role in angiogenesis and tumor metastasis, the function of CMSS1 is unclear. RNA-Seq expression data from GTEx showed a ubiquitous expression pattern of CMSS1 in 53 different human tissues. Immunofluorescence staining with a CMSS1-specific antibody showed bright nucleoli staining in A-431, U2-OS, and U-251 MG cells (The Human Protein Atlas), consistent with an RNA-binding protein. RNA-binding proteins are important for hematopoiesis. Notably, the expression levels of 6 RNA-binding proteins, including CMSS1, reliably distinguished low-risk from high-risk patients with diffuse large B-cell lymphoma (DLBCL).[5] Since dimerization is an important feature of RNA-binding proteins, it is likely that CMSS1 provides a dimerization motif for the CMSS1::FLT1 fusion protein, resulting in a ligand-independent dimerization and constitutive kinase activation of FLT1. The cellular signaling of FLT1 is not well-defined yet, however, PLCγ-PKC-MAPK signaling is required for VEGFA/KDR-induced endothelial proliferation. KDR 1175Y is phosphorylated when activated, which serves as a docking site for PLCγ. The PLC-γ then stimulates hydrolysis of phosphatidylinositol (PIP2), leading to protein kinase C (PKC) activation and subsequent raf-1/MAPK activation. Since FLT1 shares the same KDR 1175Y motif, a similar PLCγ-PKC-MAPK signaling likely contributes to the oncogenesis of CMSS1::FLT1.[6]

The activation of tyrosine kinase receptors is an important pathway implicated in the oncogenesis of myeloid neoplasms. Specifically, *FLT3* is altered in roughly a quarter of AML cases. In myeloid/lymphoid neoplasms characterized by eosinophilia, rearrangements involve *PDGFRA/B*, *FGFR1*, *JAK2*, *FLT3*, and *ABL1*. Notably, tyrosine kinase inhibitors can successfully treat patients with these genetic anomalies. Our studies have shown that cells expressing CMSS1::FLT1 are sensitive to the tyrosine kinase inhibitor Axitinib, which targets VEGFRs and PDGFRs and is FDA-approved for advanced renal cell carcinoma. Additionally, inhibitors that target the PLCγPKC-MAPK signaling pathway, such as the PKC inhibitor Midostaurin and MAPK inhibitor Ulixertinib, may also be helpful.

In summary, we reported the first documented case of cancer with VEGFR rearrangement, resulting in the activation of FLT1 signaling. Notably, these tumor cells are sensitive to the FLT1 inhibitor. Although this fusion is rare, it provides a unique opportunity to explore the downstream FLT1 signaling pathway, which plays a crucial role in angiogenesis and tumor metastasis.

AUTHOR CONTRIBUTIONS

Xiaoshan Yang, Lingfeng Liu, and Xiaojun Chen performed experiments. Liying Zhang, Bingzong Li, and Yu Sun provided CNL patient samples and clinical data. Yu Sun and Sheng Xiao analyzed the data. Sheng Xiao, Hong Zhang, and Jun Li commented on the paper. Xiaoshan Yang and Sheng Xiao wrote the paper. Yu Sun and Sheng Xiao designed the research. All authors contributed to writing the paper by providing guidance and comments on its content.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

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

Liying Zhang[1], Xiaoshan Yang[2], Xueping Ge[1], Bingzong Li[1], Hong Zhang[3], Jun Li[1], Lingfeng Liu[2], Xiaojun Chen[2], Wentao Hu[2], Yu Sun[1], Sheng Xiao[4]

[1]*Department of Hematology, The Second Affiliated Hospital of Soochow University, Suzhou, Jiangsu, China*

[2]*Suzhou Sano Precision Medicine Ltd, Suzhou, Jiangsu, China*

[3]*Clinical Laboratory of Medicine, The Second Affiliated Hospital of Soochow University, Suzhou, Jiangsu, China*

[4]*Department of Pathology, Brigham and Women's Hospital, Harvard Medical School, Boston, Massachusetts, USA*

Correspondence

Sheng Xiao, Department of Pathology, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA.
Email: sxiao@partners.org

Yu Sun, Department of Hematology, The Second Affiliated Hospital of Soochow University, Suzhou, Jiangsu, China.
Email: sunyu2084@suda.edu.cn

Liying Zhang and Xiaoshan Yang contributed equally to this work.

ORCID

Xiaoshan Yang https://orcid.org/0000-0003-1871-4645
Bingzong Li https://orcid.org/0000-0002-2732-9354

REFERENCES

1. Robinson D, Wu Y, Lin S. The protein tyrosine kinase family of the human genome. *Oncogene*. 2000;49:5548-5557.
2. Barleon B, Sozzani S, Zhou D, et al. Migration of human monocytes in response to vascular endothelial growth factor (VEGF) is mediated via the VEGF receptor Flt-1. *Blood*. 1996;87:3336-3343.
3. Nilsson MB, Giri U, Gudikote J, et al. KDR amplification is associated with VEGF-induced activation of the mTOR and invasion pathways but does not predict clinical benefit to the VEGFR TKI vandetanib. *Clin Cancer Res*. 2016;22:1940-1950.
4. Hiratsuka S, Minowa O, Kuno J, Noda T, Shibuya M. Flt-1 lacking the tyrosine kinase domain is sufficient for normal development and angiogenesis in mice. *Proc Natl Acad Sci U S A*. 1998;95:9349-9354.
5. Xie Y, Luo X, He H, Pan T, He Y. Identification of an individualized RNA binding protein-based prognostic signature for diffuse large B-cell lymphoma. *Cancer Med*. 2021;10(8):2703-2713.
6. Sakurai Y, Ohgimoto K, Kataoka Y, Yoshida N, Shibuya M. Essential role of Flk-1 (VEGF receptor 2) tyrosine residue 1173 in vasculogenesis in mice. *Proc Natl Acad Sci U S A*. 2005;102:1076-1081.