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RUNX1::MIR99AHG Chimera in Acute Myeloid Leukemia

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ABSTRACT

RUNX1 fuses with over 70 different partner genes in hematological neoplasms. While common *RUNX1* chimeras have been extensively studied and their prognosis is well established, our current understanding of less common *RUNX1* chimeras is limited. Here, we present a case of acute myeloid leukemia (AML) with a rare *RUNX1* chimera. Bone marrow cells obtained at diagnosis from a 71-year-old patient diagnosed with AML-M5 were studied using G-banding, fluorescence in situ hybridization, array comparative genomic hybridization, RNA sequencing, PCR, and Sanger sequencing. Combined findings from the abovementioned assays suggested three cytogenetic clones: one with a normal karyotype, one with inv(21)(q21q22), and one with two inv(21)(q21q22). The molecular analysis revealed the fusion of *RUNX1* with *MIR99AHG* (at 21q21.1), further supporting the presence of an inv(21)(q21q22). The present case is the third reported AML harboring a *RUNX1::MIR99AHG* chimera. Similar to the two previously described AML patients, our case also had an *FLT3* aberration.

1 | Introduction

The RUNX family transcription factor 1 gene (*RUNX1* on 21q22) encodes the DNA-binding alpha subunit of the heterodimeric core binding factor (CBF) [1, 2]. RUNX1 binds to the consensus sequence "YGYGGTY" (Y = pyrimidine) which is the core element of many enhancers and promoters [1], while the beta-subunit of CBF, encoded by the *CBFB* gene (on 16q22), enhances the DNA-binding affinity of the complex [2]. Both *RUNX1* and *CBFB* have been found to undergo rearrangements and serve as fusion partners for chimeric genes in leukemias [3].

RUNX1 has been identified as a promiscuous fusion gene, known to fuse with over 70 different partner genes in both acute myeloid leukemia (AML) and acute lymphoblastic leukemia (ALL) [3]. These fusions can occur in-frame, resulting in the production of structurally diverse chimeric proteins, or out-of-frame, leading to truncation of *RUNX1* and the coding protein [3]. Conversely, *CBFB* has been found to specifically fuse with *MYH11* in AML carrying inv(16)(p13.1q22) or t(16;16)(p13.1;q22) [3]. However, in one case of AML carrying a t(2;16)(q37;q22), a *CBFB::PPP1R7* chimera was found [3].

The prognosis of common *RUNX1* chimeras, like *RUNX1::RUNX1T1* in AML with t(8;21)(q22;q22) and *ETV6::RUNX1* in pediatric pre-B-ALL with t(12;21)(p13;q22), is well established [4, 5]. As treatment protocols for acute leukemia often depend on specific genetic alterations, there is growing clinical interest in understanding even rare *RUNX1* fusions [6]. This interest persists even in disease subgroups where targeted therapies against the leukemogenic defect are not yet available. However, our understanding of less common *RUNX1* chimeras is currently limited but steadily expanding [7]. Initially, unique genetic aberrations, such as balanced translocations or fusion

genes, may emerge and become recurrent as additional cases with the same genetic abnormality are reported. Over time, these recurrent genetic aberrations may acquire prognostic significance, providing an opportunity to explore the mechanisms of leukemogenesis in patients with these rare abnormalities. Given this context, we here present the molecular genetic and clinical features of a case of AML with an inv(21), resulting in the formation of a *RUNX1* chimera.

2 | Case Presentation

A 71-year-old male was admitted to the hospital following 3 weeks of increasing fatigue; he had been more or less bedridden for the last 4–5 days. His medical history was remarkable for hypertension, atherosclerosis, and thromboendoarterectomy of his right internal carotid artery 10 years previously. At admission, he had leukocytosis (43.4×10^9/L; ref. 3.5–10.0), anemia (hemoglobin 9.9 g/dL; ref. 13.4–17.0), neutropenia (0.7×10^9/L; ref. 1.5–7.3), and thrombocytopenia (125×10^9/L; ref. 145–390). His renal function was impaired (creatinine 158 μmol/L; ref. 60–105). A blood smear revealed that the leukocytosis was due to an increase in monocytes (56%) and monoblasts (36%). A trephine biopsy showed massive infiltration of blasts and monocytes, and flow cytometric assessment of a bone aspirate showed the following immunophenotype: $CD34^+CD13^+CD14^{(+)}CD15^-CD19^-CD33^+CD36^+CD38^+CD56^-CD64^+CD71^+CD117^+cyMPO^+$. Molecular analysis at another laboratory found a *FLT3* internal tandem duplication (ITD) with an ITD ratio of 0.57 and a *CSF3R* variant (NM_156039.3:c2442T > G; NP_724781.1:pY814*) with a variant allele frequency (VAF) of 7.8%.

Standard induction therapy for AML with daunorubicin and cytarabine was delivered. However, he succumbed to treatment toxicity before any signs of hematological reconstitution.

3 | Methods

Bone marrow cells obtained at diagnosis were cytogenetically investigated using standard methods and karyotyped according to the 2020 Guidelines of the International System for Human Cytogenomic Nomenclature.

Fluorescence in situ hybridization (FISH) analyses were performed using dual fusion probes targeting the fusion genes *RUNX1::RUNX1T1*, *CBFB::MYH11*, and *PML::RARA*; probes designed to detect del(5q), del(7q), and del(20q); and break-apart probes targeting the *KMT2A* and *RUNX1* genes. All probes were purchased from Oxford Gene Technology (OGT, Begbroke, Oxfordshire, UK). FISH analyses were performed according to the OGT's recommended protocol, and fluorescent signals were captured and analyzed using the CytoVision system (Leica Biosystems, Newcastle, UK).

Array comparative genomic hybridization (aCGH) analysis was performed using the CytoSure array products (OGT) following the company's protocols. Genomic DNA was extracted from the patient's bone marrow sample at diagnosis using a Maxwell RSC Instrument together with an RSC Whole Blood DNA Kit (Promega, Madison, Wisconsin, USA) and the concentration was measured using a Quantus fluorometer (Promega). The reference DNA was Promega's human genomic male DNA (Promega). The CytoSure Genomic DNA Labeling Kit was used for the labeling of 1 μg of patients and reference DNAs. The slides (CytoSure Cancer +SNP array, 4×180k) were scanned in an Agilent SureScan Dx microarray scanner using Agilent Feature Extraction Software (version 12.1.1.1). Data were analyzed using the CytoSure Interpret analysis software (version 4.11.36).

For RNA sequencing, total RNA was extracted and sent to the Genomics Core Facility, Norwegian Radium Hospital, Oslo University Hospital for high-throughput paired-end. The software FusionCatcher was used to find fusion transcripts.

The presence of fusion transcripts was confirmed by reverse transcription (RT), polymerase chain reaction (PCR), and Sanger sequencing. In brief, 1 μg of total RNA was reverse-transcribed, and cDNA corresponding to 20 ng total RNA was used as a template in subsequent PCR amplification using the primers RUNX1-607F1 (5′-CTA CTC GGC TGA GCT GAG AAA TGC T-3′) and MIR99AHG-520R1 (5′-AAT GCA CAG CAA TCA GTT CCT CAC A-3′). The PCR products were subsequently sequenced using the BigDye Direct Cycle Sequencing Kit (Thermo Fisher Scientific, Waltham, MA, USA). The following primer combinations were used: M13For-RUNX1-723F1 (5′-TGT AAA ACG ACG GCC AGT CTG TCT TCA CAA ACC CAC CGC A-3′)/M13Rev-MIR99AHG-213R1 (5′-CAG GAA ACA GCT ATG ACC GAA CGC AGG GTA AAA CAA TCG GA-3′); M13For-RUNX1-723F1/M13Rev-MIR99AHG-332R1 (5′-CAG GAA ACA GCT ATG ACC GTT GAC TCA TGG CCT AGG GAA AGC-3′); M13For-RUNX1-723F1/M13Rev-MIR99AHG-461R1 (5′-CAG GAA ACA GCT ATG ACC CAT TCT GAC CTC AGC CTC CTC CA-3′).

The sequences obtained by Sanger sequencing were compared with the reference sequences NM_001754.4 for *RUNX1* transcript variant 1 and NR_136549.1 for mir-99a-let-7c cluster host gene (*MIR99AHG*) transcript variant 13, long noncoding RNA using the Basic Local Alignment Search Tool (BLAST), and mapped on the Human GRCh37/hg19 assembly with the BLAST-like alignment tool (BLAT) [8–10].

4 | Results

The initial G-banding analysis of 25 examined metaphases revealed a normal male karyotype (data not shown). Subsequent FISH analyses showed no evidence of deletions of 5q, 7q, or 20q, trisomy 8, rearrangement of the *KMT2A* locus at 11q23, or any of the fusion genes *PML::RARA*, *RUNX1::RUNX1T1*, or *CBFB::MYH11* in 200 interphase nuclei studied (data not shown).

However, using the *RUNX1::RUNX1T1* dual fusion probe, 3–4 signals for *RUNX1* were observed in 168 out of 211 interphase nuclei (data not shown). Subsequent FISH analysis with a break-apart probe for the *RUNX1* gene (Figure 1A) revealed a split of one *RUNX1* locus in 32 out of 100 nuclei, a split of both *RUNX1* loci in 37 out of 100 nuclei, and a normal pattern in 31 out of 100 nuclei. Figure 1B shows an interphase nucleus with two red and two green signals of the *RUNX1* break-apart, indicating the rearrangement of both *RUNX1* loci. FISH analysis on metaphase spreads demonstrated that both parts of the split signals were located on the same

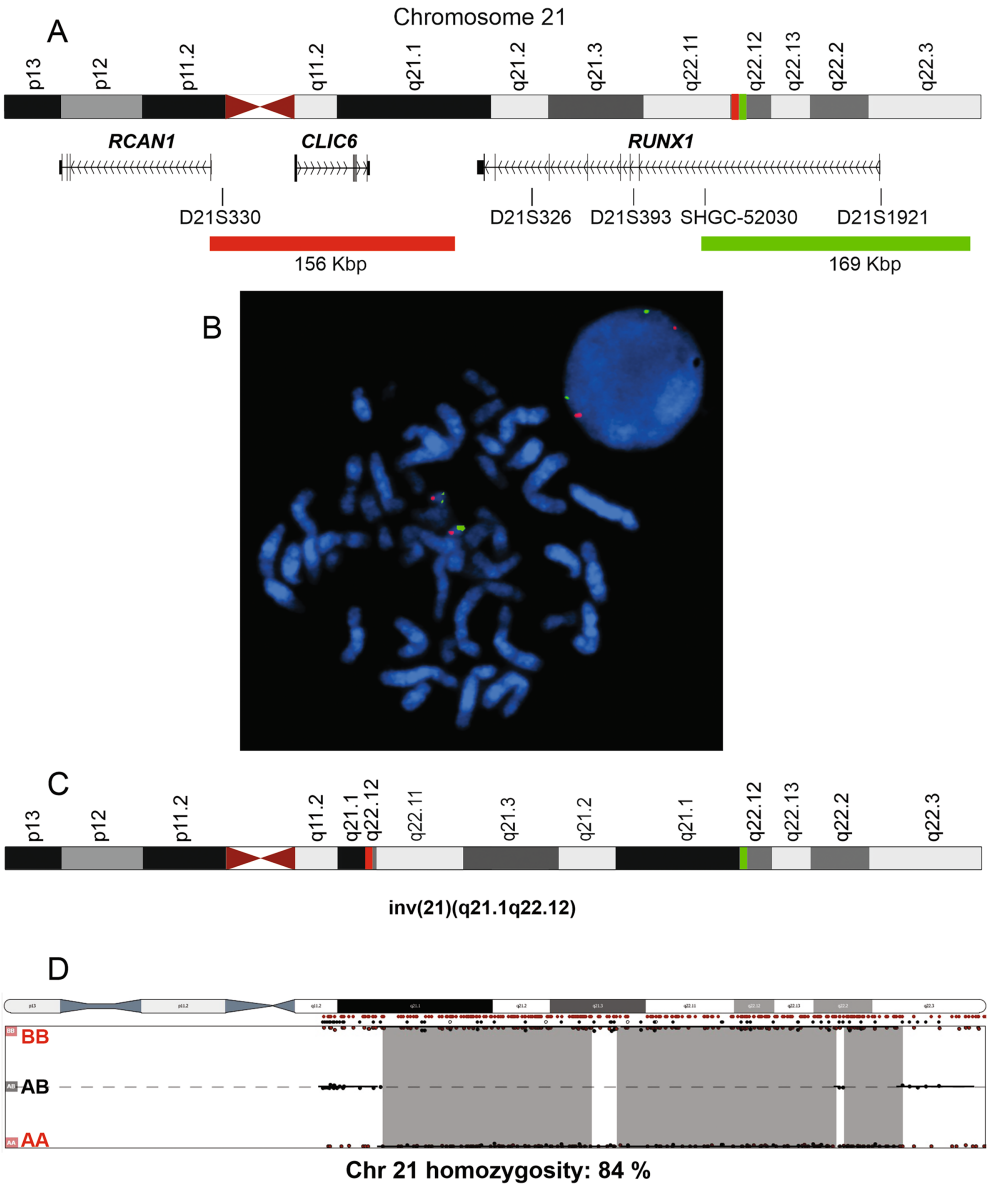

FIGURE 1 | Fluorescence in situ hybridization (FISH) of the bone marrow cells of the patient diagnosed with AML-M5. (A) *RUNX1* break-apart FISH probe. It consists of a proximal red part and a distal green part. Both parts hybridized on the sub-band 21q22.12. (B) Interphase nucleus showing two red and two green signals of the *RUNX1* break-apart probe indicating rearrangement of both *RUNX1* loci. A metaphase spread showed that the red signals were hybridized close to the centromere of chromosome 21, indicating the presence of an inv(21). (C) Ideogram of the inv(21)(q21.1q22.12) together with the hybridization areas of the red and green parts of the *RUNX1* break-apart probe. (D) Ideogram of chromosome 21 showing the distribution of single nucleotide polymorphisms (SNPs). Homozygous SNPs (AA or BB) are represented by red dots, and heterozygous SNPs (AB) are indicated by black dots. In total 339 SNPs were identified along chromosome 21. Of these, 282 SNPs were homozygous (AA or BB), 53 SNPs were heterozygous (AB), and 4 SNPs had an unknown genotype. The detected homozygosity across chromosome 21 was 84%.

chromosome 21, indicating an intrachromosomal rearrangement involving the *RUNX1* gene. Figure 1B also shows a metaphase spread in which the red signals were hybridized close to the centromere of both chromosomes 21, indicating the presence of two inv(21) in the metaphase spread. Based on these metaphase FISH findings, an inversion of chromosome 21 was suspected, with one breakpoint located within the *RUNX1* gene (Figure 1C).

The aCGH analysis did not detect any gains or losses of genetic material (data not shown). The B allele frequency methodology revealed 60%–68% homozygosity for chromosomes 1–20, and 22 (data not shown). Chromosome 21 exhibited higher levels of homozygosity at 84% (Figure 1D). The high level of SNP homozygosity on chromosome 21 suggested an acquired uniparental disomy (aUPD) of chromosome 21.

RNA sequencing analysis using the FusionCatcher software identified four *RUNX1::MIR99AHG* chimeric transcripts (Figure 2A). Three of these transcripts resulted from fusions between exon 6 of the *RUNX1* transcript variant 1 (NM_001754.4) and exons 2, 4, or 3 of *MIR99AHG* transcript variant 13 (NR_136549.1) (Figure 2A,B). In the fourth chimeric transcript, exon 6 of *RUNX1* fused to a sequence approximately 1.4 kbp upstream of exon 1 of *MIR99AHG* (Figure 2A,B). These chimeric transcripts are predicted to encode

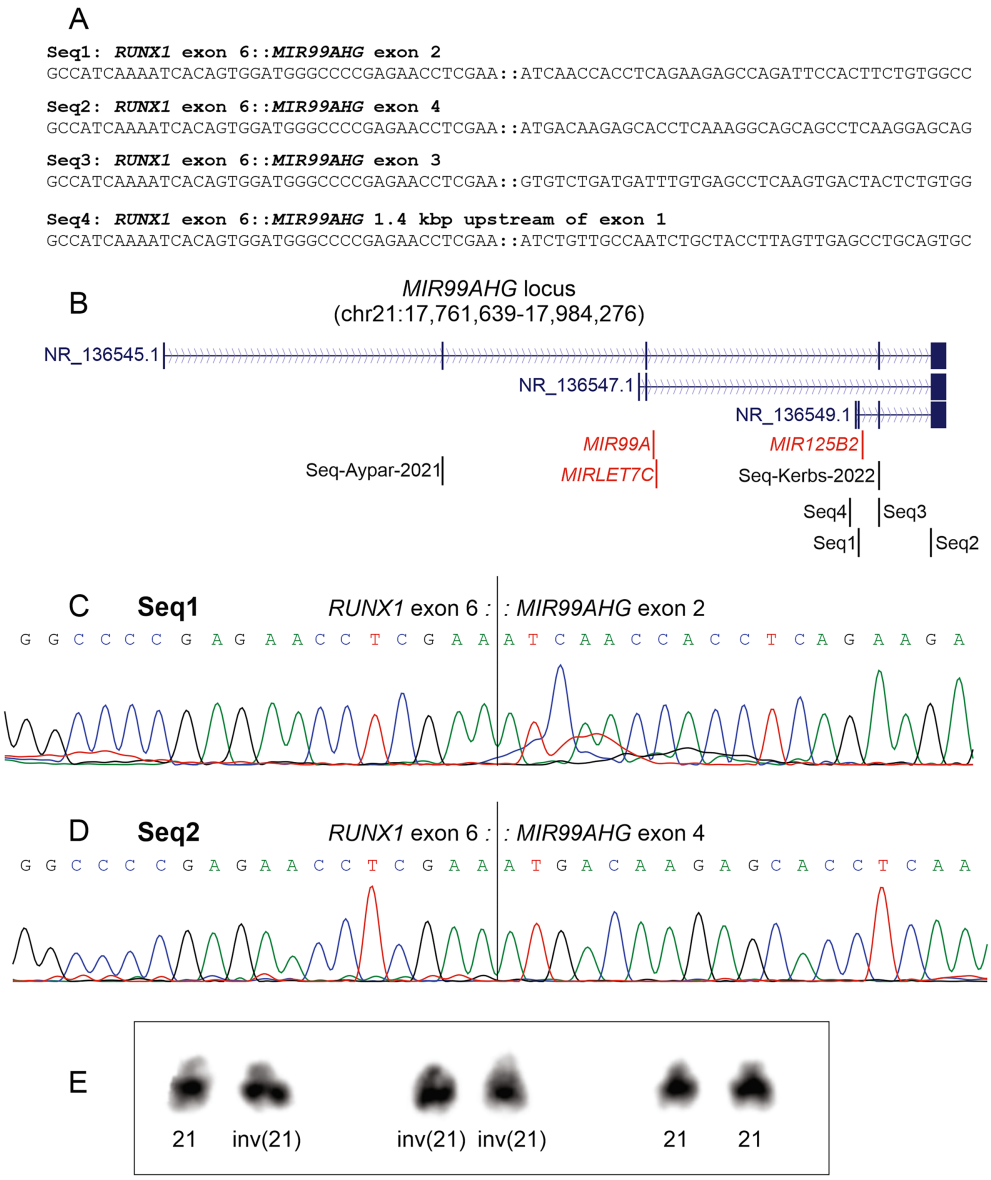

FIGURE 2 | Detection of *RUNX1::MIR99AHG* chimera and inv(21) in bone marrow cells from the patient diagnosed with AML-M5. (A) RNA sequencing analysis using FusionCatcher identified the presence of four *RUNX1::MIR99AHG* chimeric transcripts. Exons are based on the reference sequences NM_001754.4 for RUNX1 transcript variant 1 and NR_136549.1 for MIR99AHG transcript variant 13. (B) Genomic view of the *MIR99AHG* locus on 21q21.1, including transcripts NR_136545.1, NR_136547.1, and NR_136549.1, along with *MIR99A*, *MIRLET7C*, *MIR125B2*, and reported breakpoints. (C) Partial sequence chromatogram illustrating the fusion of *RUNX1* exon 6 with *MIR99AHG* exon 2. (D) Partial sequence chromatogram illustrating the fusion of *RUNX1* exon 6 with *MIR99AHG* exon 4. (E) Partial karyograms showing one normal chromosome 21 and one inv(21)(q21q22), two inv(21)(q21q22), and two normal chromosomes 21.

truncated RUNX1 proteins comprising the first 204 amino acid residues of RUNX1 (NP_001745.2), along with additional amino acids contributed by the fused sequences from *MIR99AHG*. Specifically, fusion with exon 2 adds 60 amino acids (NQPPQK SQIPLLWPGLLHYGWSDCFTLRSMLLNLGSFLKCLMICE PQVTTLWNHLSLPLN), fusion with exon 4 adds 26 amino acids (NDKSTSKAAASRSSHGPRLVARMQKT), fusion with exon 3 adds 2 amino acids (SV), and fusion with the upstream sequence of exon 1 adds 7 amino acids (ACSAALH).

RT-PCR followed by direct cycling Sanger sequencing confirmed the presence of the fusion between exon 6 of *RUNX1* and exons 2 and 4 of *MIR99AHG* (Figure 2C,D). No other chimeric transcripts were examined.

Because of the findings from the FISH and CGH analyzes, as well as the presence of *RUNX1::MIR99AHG* chimeric transcripts, 25 metaphases of G-banded chromosomes were re-examined. This new examination revealed eight metaphases with one inv(21)(q21q22), ten metaphases with two inv(21)(q21q22), and seven metaphases with a normal karyotype (Figure 2E). Consequently, the revised karyotype of the examined bone marrow leukemic cells was: 46,XY,inv(21)(q21q22)[8]/46,XY, inv(21)(q21q22)×2[10]/46,XY[7].

5 | Discussion

We present a case of AML in which combined findings from G-banding, FISH, aCGH, and RNA sequencing assays showed that the bone marrow leukemic cells carried an inv(21)(q21q22), resulting in the fusion of the *RUNX1* gene (21q22) with the *MIR99AHG* gene at 21q21.2 (Figures 1 and 2). To the best of our knowledge, this is the third reported AML case where *RUNX1::MIR99AHG* chimera has been identified (Table 1) [7, 11]. The first patient was a 23-year-old woman with the karyotype of 46,XX,inv(21)(q11.2q22)[8]/46,idem,del(1)(p36.1p36.3),add(20)(q13.3)[1]/46,XX[11] and a *FLT3*-ITD (36 and 78 bp) in a small fraction of leukemic cells (VAF 4%). In the *RUNX1::MIR99AHG* transcript, exon 8 of *RUNX1* fused to exon 5 of the reference sequence NR_027790, corresponding to transcript variant 1 of *MIR99AHG* (Figure 2B) [7]. The second patient was a 42-year-old man (patient SIH172) diagnosed with AML-M5. The karyotype was 43–45,XY,−18[cp4]/46,XY. He had FLT3 p.D835Y (21%), SETD2 p.P241A (45%), and also carried a *RUNX1::MIR99AHG* chimera. The fusion positions between *RUNX1* and *MIR99AHG* were not provided [11]. Our patient, similar to the first patient, also carried a *FLT3*-ITD. However, the fusion points in the *RUNX1::MIR99AHG* chimeric transcripts were different (Table 1) [7]. The fusion occurred between exon 6 of *RUNX1* (NM_001754.4) and exons 2, 4, or 3 of *MIR99AHG* transcript variant 13 (NR_136549.1) or with a sequence approximately 1.4 kbp upstream of exon 1 of *MIR99AHG* (Figure 2A,B). These *RUNX1::MIR99AHG* transcripts are expected to encode truncated RUNX1 peptides that retain the Runt homology domain (RHD), crucial for both heterodimerization with CBFB and DNA binding [12], while lacking the C-terminal part of the protein. The C-terminal region contains the transcriptional activation domain as well as sequences critical for interactions with other proteins and for appropriate subnuclear targeting of RUNX1 [13]. Previous studies have demonstrated that such truncated RUNX1 proteins function as inhibitors of the normal RUNX1 protein and may contribute to leukemogenesis [14–17]. In a patient with AML carrying a t(19;21)(q13;q22) translocation, *RUNX1* exon 6 fused with an intergenic sequence from chromosome 19 (GenBank: AY004251.2). This fusion transcript resulted in a truncated RUNX1 protein that included the first 204 amino acids of the normal RUNX1 protein (NP_001745.2), along with 17 additional amino acids from the chromosome 19 sequence (SCLQIMKPVNQDRPISL) [14]. In vitro assays demonstrated that this truncated protein inhibited the normal RUNX1 protein's function [14]. A similar inhibitory effect was observed in an AML with a t(12;21)(q12;q22), where the fusion of *RUNX1* exon 7 with an intergenic sequence at 12q12 also produced a truncated RUNX1 protein [15]. In an MDS/AML patient with a t(1;21)(p32;q22), a chimeric transcript was identified in which *RUNX1* exon 6 fused with an intronic sequence from the *NDC1* gene (GenBank: KF305770) [16]. This fusion produced a truncated RUNX1 protein consisting of the first 204 amino acids of RUNX1 and 11 additional amino acids from the *NDC1* intronic sequence (NSLTWPRYPHI). In vitro studies with transduced human hematopoietic progenitor/stem cells showed that this truncated RUNX1 protein increased cell proliferation and self-renewal while disrupting normal differentiation, likely by interfering with the AML1b isoform [16]. In a case of relapsed AML, a rearrangement between 6q16 and 21q22 produced multiple chimeric transcripts, where *RUNX1* exon 6 or 7 fused with the antisense strand of the *GRIK2* gene [17]. These chimeric transcripts encoded truncated RUNX1 proteins that promoted the proliferation of 32D myeloid leukemia cells in response to *CSF3* and induced the expression of *CSF3R* [17]. Taking all the abovementioned data into consideration, the truncated RUNX1 protein appears to be a contributing factor in leukemogenesis due to the absence of its C-terminal region, which contains the subnuclear targeting and transactivation domains.

MIR99AHG is a long noncoding (lnc) RNA gene (Gene ID: 388815, HGNC:1274) that transcribes 16 different alternatively spliced RNA molecules. It belongs to microRNA noncoding host genes, which are defined as lncRNAs containing a microRNA

TABLE 1 | Acute myeloid leukemias were reported to carry the *RUNX1::MIR99AHG* chimera.

Case	Sex/age (years)	Reported karyotype	Additional genetic abnormalities	***RUNX1* fusion point (mapped to the GRCh37/hg19 human genome assembly)**	***MIR99AHG* fusion point (mapped to the GRCh37/hg19 human genome assembly)**	References
1	F/23	46,XX,inv(21)(q11.2q22)[8]/46,idem,del(1)(p36.1p36.3),add(20)(q13.3)[1]/46,XX[11]	NP_724781.1(CSF3R):p.(Q776*) *FLT3*-ITD	Chr21:36171598:−	Chr21:17859810:+	[7]
2	M/42	43–45,XY,−18[cp4]/46,XY	NP_004110.2(FLT3):p.(D835Y) NP_054878.5(SETD2):p.(P241A)	Not available	Not available	[11]
3	M/71	46,XY,inv(21)(q21q22)[8]/46,XY,inv(21)(q21q22)×2[10]/46,XY[7].	NP_724781.1(CSF3R):p.(Y814*) *FLT3*-ITD	Chr21:36231771:− Chr21:36231771:− Chr21:36231771:− Chr21:36231771:−	Chr21:17961673:+ Chr21:17979314:+ Chr21:17966660:+ Chr21:17959511:+	Present study

(MIR) gene in either an intron or an exon on the same strand. The *MIR99AHG* gene encompasses the *MIR99A* (NR_029514.1), *MIRLET7* (NR_029480.1), and *MIR125B2* (NR_029694.1) in intron 6 of the transcript variant 1 (NR_027790.3) (Figure 2B). These three *MIR* genes are expressed as a tricistronic message (*MIR99A*/*MIRLET7*/*MIR125B2*) originating from a single transcript, which also includes the lincRNA host genes [18, 19]. In hematopoiesis, the tricistronic *MIR99A/MIRLET7/MIR125B2* message is primarily expressed in CD34+ hematopoietic stem and progenitor cells (HSPCs), erythroid cells, and CD4+ T cells. The tricistronic message has also been detected in acute megakaryoblastic leukemia, where it acts as an oncogene promoting disease progression [18, 19]. A significant upregulation of *MIR99AHG* resulting from the *RUNX1::MIR99AHG* chimera has been reported [11].

A recurrent chimera between the nuclear receptor-interacting protein 1 (*NRIP1*) gene and *MIR99AHG* has recently been identified in AML (*NRIP1::MIR99AHG*), believed to result from a putative inv(21)(q11.2;q21.1) [11, 20]. In *NRIP1::MIR99AHG*, the noncoding exon 3 of *NRIP1* (NM_003489.4) fused with exon 3 of NR_136549.1 [20], resulting in elevated expression of the 3′-end partner *MIR99AHG* [11, 20]. The fusion breakpoints within *MIR99AHG* occurred approximately 4 kbp distal to *MIR125B2*, leading to *NRIP1::MIR99AHG* without the presence of the tricistronic message (*MIR99A/MIRLET7/MIR125B2*) (Figure 2B) [20]. In the present AML case, the chimeric *RUNX1::MIR99AHG* transcripts indicated a possible disruption of the tricistronic gene cluster (Figure 2A,B). However, in the previously reported *RUNX1::MIR99AHG* transcript [7], the breakpoint within *MIR99AHG* occurred approximately 50 kbp proximal to *MIR99A* and thus did not disrupt the tricistronic gene cluster. Current data are insufficient to draw any conclusions about the importance of the presence, absence, or disruption of the tricistronic message (*MIR99A/MIRLET7/MIR125B2*) in AML carrying *NRIP1::MIR99AHG* or *RUNX1::MIR99AHG* chimeras.

The aCGH data revealed an aUPD of chromosome 21, accompanied by an inversion inv(21)(q21q22) and a *RUNX1::MIR99AHG* chimera. G-banding, karyotyping, and FISH examination indicated that the aUPD was present in approximately 40% of the bone marrow cells. This observation is consistent with the known association between aUPD and homozygous genetic alterations in AML [21]. Furthermore, it supports previously published findings that aUPDs involving chromosome 21 include *RUNX1* and are associated with homozygous genetic alterations in *RUNX1* [22, 23].

In conclusion, we here describe a patient diagnosed with AML-M5 harboring a *RUNX1::MIR99AHG* chimera along with an *FLT3*-ITD. Our data further underscore the recurrence of the *RUNX1::MIR99AHG* chimera in AML and its potential association with *FLT3* genetic alterations.

Ethics Statement

The study was approved by The Regional Committee for Medical and Health Research Ethics South East Norway, approval number 2010/1389/REK sør-øst A. All patient information has been de-identified.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

All available data are included in the manuscript and its figures.

References

1. I. N. Melnikova, B. E. Crute, S. Wang, and N. A. Speck, "Sequence Specificity of the Core-Binding Factor," *Journal of Virology* 67, no. 4 (1993): 2408–2411.

2. Q. Wang, T. Stacy, J. D. Miller, et al., "The CBFbeta Subunit Is Essential for CBFalpha2 (AML1) Function In Vivo," *Cell* 87, no. 4 (1996): 697–708.

3. F. Mitelman, B. Johansson, and F. Mertens, "Mitelman Database of Chromosome Aberrations and Gene Fusions in Cancer," (2024).

4. K. H. Metzeler and C. D. Bloomfield, "Clinical Relevance of RUNX1 and CBFB Alterations in Acute Myeloid Leukemia and Other Hematological Disorders," *Advances in Experimental Medicine and Biology* 962 (2017): 175–199.

5. G. Rodriguez-Hernandez, D. Schafer, A. Gavilan, et al., "Modeling the Process of Childhood ETV6-RUNX1 B-Cell Leukemias," *Oncotarget* 8, no. 60 (2017): 102674–102680.

6. F. Gonzales, A. Barthelemy, P. Peyrouze, et al., "Targeting RUNX1 in Acute Myeloid Leukemia: Preclinical Innovations and Therapeutic Implications," *Expert Opinion on Therapeutic Targets* 25, no. 4 (2021): 299–309.

7. U. Aypar, J. Yao, D. M. Londono, et al., "Rare and Novel *RUNX1* Fusions in Myeloid Neoplasms: A Single-Institute Experience," *Genes, Chromosomes & Cancer* 60, no. 2 (2021): 100–107.

8. S. F. Altschul, W. Gish, W. Miller, E. W. Myers, and D. J. Lipman, "Basic Local Alignment Search Tool," *Journal of Molecular Biology* 215, no. 3 (1990): 403–410.

9. W. J. Kent, "BLAT—The BLAST-Like Alignment Tool," *Genome Research* 12, no. 4 (2002): 656–664.

10. W. J. Kent, C. W. Sugnet, T. S. Furey, et al., "The Human Genome Browser at UCSC," *Genome Research* 12, no. 6 (2002): 996–1006.

11. W. Y. Cheng, J. F. Li, Y. M. Zhu, et al., "Transcriptome-Based Molecular Subtypes and Differentiation Hierarchies Improve the Classification Framework of Acute Myeloid Leukemia," *Proceedings of the National Academy of Sciences of the United States of America* 119, no. 49 (2022): e2211429119.

12. E. Ogawa, M. Maruyama, H. Kagoshima, et al., "PEBP2/PEA2 Represents a Family of Transcription Factors Homologous to the Products of the Drosophila Runt Gene and the Human AML1 Gene," *Proceedings of the National Academy of Sciences of the United States of America* 90, no. 14 (1993): 6859–6863.

13. C. R. Dowdy, R. Xie, D. Frederick, et al., "Definitive Hematopoiesis Requires Runx1 C-Terminal-Mediated Subnuclear Targeting and Transactivation," *Human Molecular Genetics* 19, no. 6 (2010): 1048–1057.

14. R. Hromas, T. Busse, A. Carroll, et al., "Fusion AML1 Transcript in a Radiation-Associated Leukemia Results in a Truncated Inhibitory AML1 Protein," *Blood* 97, no. 7 (2001): 2168–2170.

15. H. Ramsey, D. E. Zhang, K. Richkind, A. Burcoglu-O'Ral, and R. Hromas, "Fusion of AML1/Runx1 to Copine VIII, a Novel Member of the Copine Family, in an Aggressive Acute Myelogenous Leukemia With t(12;21) Translocation," *Leukemia* 17, no. 8 (2003): 1665–1666.

16. S. Rodriguez-Perales, R. Torres-Ruiz, J. Suela, et al., "Truncated RUNX1 Protein Generated by a Novel t(1;21)(p32;q22) Chromosomal

Translocation Impairs the Proliferation and Differentiation of Human Hematopoietic Progenitors," *Oncogene* 35, no. 1 (2016): 125–134.

17. A. Abe, Y. Yamamoto, A. Katsumi, et al., "Truncated RUNX1 Generated by the Fusion of *RUNX1* to Antisense *GRIK2* via a Cryptic Chromosome Translocation Enhances Sensitivity to Granulocyte Colony-Stimulating Factor," *Cytogenetic and Genome Research* 160, no. 5 (2020): 255–263.

18. S. Emmrich, M. Rasche, J. Schoning, et al., "miR-99a/100~125b Tricistrons Regulate Hematopoietic Stem and Progenitor Cell Homeostasis by Shifting the Balance Between TGFbeta and Wnt Signaling," *Genes & Development* 28, no. 8 (2014): 858–874.

19. S. Emmrich, A. Streltsov, F. Schmidt, V. R. Thangapandi, D. Reinhardt, and J. H. Klusmann, "LincRNAs MONC and MIR100HG Act as Oncogenes in Acute Megakaryoblastic Leukemia," *Molecular Cancer* 13 (2014): 171.

20. P. Kerbs, S. Vosberg, S. Krebs, et al., "Fusion Gene Detection by RNA-Sequencing Complements Diagnostics of Acute Myeloid Leukemia and Identifies Recurring NRIP1-MIR99AHG Rearrangements," *Haematologica* 107, no. 1 (2022): 100–111.

21. J. Fitzgibbon, L. L. Smith, M. Raghavan, et al., "Association Between Acquired Uniparental Disomy and Homozygous Gene Mutation in Acute Myeloid Leukemias," *Cancer Research* 65, no. 20 (2005): 9152–9154.

22. M. Gupta, M. Raghavan, R. E. Gale, et al., "Novel Regions of Acquired Uniparental Disomy Discovered in Acute Myeloid Leukemia," *Genes, Chromosomes & Cancer* 47, no. 9 (2008): 729–739.

23. C. J. Walker, J. Kohlschmidt, A. K. Eisfeld, et al., "Genetic Characterization and Prognostic Relevance of Acquired Uniparental Disomies in Cytogenetically Normal Acute Myeloid Leukemia," *Clinical Cancer Research* 25, no. 21 (2019): 6524–6531.