Molecular Genetic Characterization of Acute Myeloid Leukemia With Trisomy 4 as the Sole Chromosome Abnormality

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Abstract. Background/Aim: The aim of the study was to determine the genetic and molecular consequences of trisomy 4, a recurrent but rare chromosomal abnormality in acute myeloid leukemia (AML). Materials and Methods: Interphase fluorescence in situ hybridization, reverse transcriptase-quantitative polymerase chain reaction for 28 chromosomal gene translocations/fusion genes, and targeted sequencing analyses were performed on five AMLs with trisomy 4 as the sole chromosomal anomaly. Results: An NPM1 frameshift mutation was found in all leukemic bone marrows, DNMT3A, FLT3, and IDH1 mutations were found in three, KIT and NRAS mutations in two, whereas IDH2 (R140Q), RUNX1, and WT1 mutations were found in only one patient each. The three patients with a DNMT3A (R882H) mutation have died. In contrast, the two patients whose leukemic cells were without this mutation, are alive 55 and 31 months after diagnosis, respectively. Conclusion: The results suggest a possible association between trisomy 4 and additional mutations that may influence prognosis.

Acute myeloid leukemia (AML) is a heterogenous group of malignancies with different clinical phenotypes and responses to therapy (1, 2). AMLs can be classified according to distinct cytogenetic and genetic abnormalities of leukemic cells at diagnosis, but also by phenotypic and epigenetic differences that act as a guide to risk assessment and choice of treatment (1, 2). AML cases with a single trisomy constitute a heterogenous subgroup with regard to clinical, morphological, and immunophenotypic features, as well as the mutational profiles (3).

Trisomy 4 is a recurrent, but rare chromosomal abnormality in AML. It may be the sole aberration or one of several chromosomal changes (4). Trisomy 4 was first described by Mecucci et al. in 1986, and a year later by Sandberg et al. (5), as the sole chromosomal abnormality in AML (6). In the Mitelman Database of Chromosome Aberrations and Gene Fusions in Cancer, 83 (48 female and 35 male patients) out of 18029 AML-cases had trisomy 4 as the sole chromosomal abnormality (http://cgap.nci.nih.gov-Chromosomes/Mitelman, database last updated on February 20, 2017). Most cases are morphologically classified as M1, M2, or M4 using the French-American-British (FAB) classification.

Because trisomy 4 is rare, few studies have addressed its clinical impact. As a consequence, the prognosis of AML with solitary trisomy 4 remains unclear (7, 8). However, a recent study based on 87 AML cases with trisomy 4 concluded that the prognostic significance of the aberration depended on the patient’s age, and despite an initial good response to treatment, patients with trisomy 4 were prone to relapse (4).

Recently, we reported the genetic, molecular and clinical features of a pediatric AML case with trisomy 4 and an FLT3-ITD mutation (9). Fluorescence in situ hybridization assay demonstrated that part of the RUNX1 probe had moved to chromosome band 6q25 indicating a cryptic t(6;21)(q25;q22) translocation. Molecular analysis of the translocation showed fusion of RUNX1 with an intergenic sequence from 6q25, resulting in a putative RUNX1 truncated protein that would contain the Runt homology domain responsible for both heterodimerization with CBFB and DNA binding (9). The findings prompted us to investigate additional cases of myeloid
malignancies with trisomy 4 as the sole karyotypic anomaly, for submicroscopic or molecular aberrations.

Materials and Methods

Patients and samples. Permission to perform the study was granted by the Regional Committee for Medical and Health Research Ethics of South-East Norway, and written informed consent was obtained from the patients.

Methods. Interphase fluorescence in situ hybridization (FISH) was performed on these samples with the CytoCell multiprobe AML/MDS panel (CytoCell, http://www.cytocell.co.uk/) looking for del(5q), PML/RARA, del(17p) (TP53), RUNX1/RUNX1T1, trisomy 8, KMT2A (MLL) splitting, -7/del(7q), CBFB/MYH11, and del(20q). The HemaVision 28Q reverse transcription-quantitative polymerase chain

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Table I. Clinicopathological and hematological findings of AML patients with trisomy 4 as the sole chromosome abnormality.

| Patient | Gender/age at diagnosis | Status | Diagnosis | Co-morbidity | Blasts (bone marrow) | Clinical characteristics | Peripheral blood | Treatment | Complete remission |
|---------|-------------------------|--------|-----------|--------------|----------------------|------------------------|-----------------|-----------|-------------------|
| 1       | F/62                    | Dead/1 week after diagnosis | AML - with dysplastic changes | Asthma, fibromyalgia, smoking, COPD, hypothyreosis. | 40%, Auer rods seen | Fatigue, fever | Hb 8.9 g/dL, WBC 62.8 ×10⁹/L, PLT 91 ×10⁹/L | 1 Induction course; cerubidine + cytarabine (3+7) | 3 consolidation courses; high dose cytarabine. | Dead before achieving CR |
| 2       | F/54                    | Dead/15 months after diagnosis | AML, FAB M2 | Asthma, depression, smoking, hyperlipidemia, COPD | 72% | Fatigue, fever, hepatomegaly | Hb 10.3 g/dL, WBC 44×10⁹/L, PLT 197 ×10⁹/L | 1 Induction course; cerubidine + cytarabine (3+7) | 3 consolidation courses; high dose cytarabine. | CR following induction; relapse after 11 months of CR |
| 3       | M/53                    | Alive/55 months | AML, FAB M1 | Hypertension, hyperlipidemia, tumor ani, squamous cell hyperplasia | 75%, Auer rods seen | Oral aphaetae | Hb 9.8 g/dL, WBC 8.34×10⁹/L, PLT 107×10⁹/L | 1 Induction course; cerubidine + cytarabine (3+7), elderly. | 1st consolidation course; mitoxantrone day 1-3, cytarabine day 1-5 + CNS prophylaxis with "triple" intrathecal. | CR following induction; still in remission |
| 4       | M/75                    | Alive/31 months | AML, FAB M4 | Bipolar disease (Lithium, lamictal), hypertension | 75%, Auer rods seen | No specific symptoms | Hb 12.8 g/dL, WBC 1.24×10⁹/L, PLT 108×10⁹/L | 1 Induction course; cerubidine + cytarabine (3+7), elderly. | 2nd consolidation course; mitoxantrone day 1-3 + cytarabine day 1-4. | CR following induction; still in remission |
| 5       | M/67                    | Dead/8 months after diagnosis | AML, FAB M2 | | 93% | Fatigue, malaise, swelling left triceps surae. | Hb 11.8 g/dL, WBC 59×10⁹/L, PLT 145 ×10⁹/L | 1 Induction + 1st consolidation course; cerubidine + cytarabine (3+7) | 2nd consolidation course; mitoxantrone day 1-3 + cytarabine day 1-4. | Allogenic stem cell transplantation, sibling donor, reduced intensity conditioning regimen | CR following induction; relapse after 7 months of CR |

A ML: Acute myeloid leukemia; COPD: chronic obstructive pulmonary disease; Hb: hemoglobin; PLT: platelet count; WBC: white blood cell count; CR: complete remission.
respectively. The leukemic cells from the deceased patients were annotated by VariantStudio v.2.2. Reporter Software v.2.6.3.2 (Illumina). SNV’s and indels < 60 bp variant calling were performed against GRCh37/hg19 with MiSeq Myeloid Sequencing Panel covers 15 full genes (exons only) and 39 additional genes that are oncogenic hotspots. Alignment and variant calling were performed against GRCh37/hg19 with MiSeq Reporter Software v.2.6.3.2 (Illumina). SNV’s and indels < 60 bp were annotated by VariantStudio v.2.2.

Results

Neither interphase FISH analyses found any aberrations nor HemaVision 28Q RT-qPCR–KIT analysis detected any of the most common AML-specific fusion transcripts.

Targeted sequencing showed that all five examined AML samples carried mutations in addition to trisomy 4 (Table II). Samples from patients 1, 2, 4, and 5 harbored four mutations whereas patient 3 had seven mutations. NPM1 frameshift mutation was found in all patients, whereas KIT and NRAS mutations were found in two patients. DNMT3A, FLT3, and IDH1 mutations were found in three patients, whereas IDH2 (R140Q), RUNX1, CUX1, KRAS, and WT1 mutations were found in one patient each. Patients 1, 2, and 5, whose AMLs showed a DNMT3A (R882H) mutation, have died. In contrast, patients 3 and 4, whose leukemic cells did not carry this mutation, are alive 55 and 31 months after diagnosis, respectively. The leukemic cells from the deceased patients 2 and 5 also harbored the KIT D816V mutation, whereas patients 3 and 4 without this mutation are alive 55 and 31 months after diagnosis. Patient 1, also deceased, did not have the acquired KIT D816V mutation. This patient succumbed suddenly probably due to a cardiac event rather than AML.

Discussion

The genetic and molecular consequences of trisomy 4 are, in general, unknown (3, 4, 7, 8). Possible mechanisms involve global gene expression alterations because of gene dosage effects, including duplication of rearranged or mutated genes on chromosome 4. No studies have as yet specifically examined general expression patterns in AML with trisomy 4. Instead, analyses have focused on KIT at 4q12, which encodes a receptor tyrosine kinase related to FLT3. Ferrari et al. (10) reported overexpression of KIT in one case with trisomy 4 as compared to AML without this abnormality, suggesting a possible pathogenetic role. Beghini et al. (11) showed that trisomy 4 resulted in duplication of a mutated KIT allele in two out of six AML cases with trisomy 4 as the sole change. Similarly, Schnittger et al. (12) reported KIT mutations in two cases with trisomy 4. On the other hand, Bains et al. (7) studied 13 AML cases with isolated trisomy 4 finding a KIT mutation in only one of them, and concluded that trisomy 4 was not generally associated with KIT mutations. Instead, FLT3 and NPM1 mutations were found in 50% and 40% of the cases, respectively.

In the present study, targeted sequencing showed concurrent multiple mutations in all patients (Table II). An NPM1 frameshift mutation was found in all leukemic bone marrows. NPM1 mutations are common in AML and are found in 20-30% of the cases (13). In addition, mutations were found in genes which are involved in the regulation of DNA methylation (DNMT3A, IDH1 and IDH2) and genes involved in cell signaling (FLT3, NRAS and KIT). RUNX1, CUX1, KRAS, and WT1 mutations were found in one patient each.

The three patients with a DNMT3A (R882H) mutation have died. In contrast, the two patients whose leukemic cells did not carry a DNMT3A mutation, are alive 55 and 31 months after diagnosis, respectively.

DNMT3A mutations are the most frequent recurrent gene mutations in AML after NPM1 and FLT3 mutations (14). The prognostic significance of DNMT3A mutations is thought to be adverse. DNMT3A mutations arise early in AML evolution and may persist during remission (1, 14). There is, however, no clear information today as to whether

Table II. Mutational profile after exome sequencing of AML patients with trisomy 4 as the sole chromosome abnormality.

| Genes   | Patient 1 | Patient 2 | Patient 3 | Patient 4 | Patient 5 |
|---------|-----------|-----------|-----------|-----------|-----------|
| NPM1    | W288fs*12 | W288fs*12 | W288fs*12 | W288fs*12 | W288fs*12 |
| DNMT3A  | R882H     | R882H     | G12S      | E598DYVDREYE | R882H     |
| NRAS    | G12D      | G12S      | D835Y     | D816V     | D816V     |
| FLT3    | D835Y     | D816V     | R132H     | R132H     | R132L     |
| KIT     | D815V     | R132H     | R140Q     | R140Q     | R140Q     |
| IDH1    | IDH2      | IDH2      | RUNX1     | RUNX1     | RUNX1     |
| IDH2    | R140Q     | E223G     | E223G     | E223G     | E223G     |
| RUNX1   | E223G     | D464N     | D464N     | D464N     | D464N     |
| CUX1    | T58I      | S480G     | T58I      | S480G     | S480G     |
| KRAS    | S480G     | S480G     | S480G     | S480G     | S480G     |

reaction (RT-qPCR–KIT) for 28 chromosome translocations/ fusion genes (DNA Diagnostics, Risskov, Denmark) was used to detect the most common AML-specific fusion transcripts. The Illumina’s TruSight Myeloid Sequencing Panel (Illumina, San Diego, CA, USA) was used to identify somatic mutations in the samples. According to the company’s information, the TruSight Myeloid Sequencing Panel covers 15 full genes (exons only) and 39 additional genes that are oncogenic hotspots. Alignment and variant calling were performed against GRCh37/hg19 with MiSeq Reporter Software v.2.6.3.2 (Illumina). SNV’s and indels < 60 bp were annotated by VariantStudio v.2.2.

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any particular relationship exists between trisomy 4 and mutations of DNMT3A, let alone whether any such combination impacts prognosis.

It is unclear whether the poor outcome for patients 1, 2, and 5 was influenced by additional mutation(s) in DNMT3A, KIT, IDH1 or a combination of these changes. The results, nevertheless, suggest a possible association between trisomy 4 and additional mutations that may affect prognosis. Novel techniques, such as targeted sequencing used in this study, may help identify the critical genes and understand their role in leukemogenesis and their influence on prognosis. Such investigations in large groups of patients are needed to collect quality data for this purpose.

Conflicts of Interest

The Authors declare that they have no potential conflicts of interest.

Authors’ Contributions

ST made hematological evaluations, treated patients, designed the research, and wrote the manuscript. LG interpreted the cytogenetics and FISH data. SH interpreted the cytogenetics and FISH data and wrote the manuscript. GET made hematologic evaluations, treated patients and wrote the manuscript. SS made hemopathologic evaluations and interpreted the targeted sequencing data. BR performed targeted sequencing and interpreted the data. HTTT made hemato logic evaluations, treated patients. IP designed and supervised the research, interpreted the data and wrote the manuscript.

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