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NUP98-PHF23 fusion is recurrent in acute myeloid leukemia and shares gene expression signature of leukemic stem cells

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ABSTRACT

Chromosome translocations involving nucleoporin 98 gene (NUP98) have been identified in a wide array of hematologic malignancies, and the resulting NUP98-associated fusions are known to play a critical role in leukemogensis through dysregulation of gene expression. Although NUP98-associated fusions were initially thought to be rare, application of molecular technologies has revealed that cryptic translocations involving NUP98 are more frequent than previously appreciated. We report an additional case of t(11;17)(p15;p13) resulting in the fusion of NUP98 and plant homeodomain finger 23 (PHF23) in a pediatric patient with acute myeloid leukemia (AML). Using RNA sequencing, we determined in-frame fusion points and also analyzed the gene expression profile of NUP98-PHF23 positive AML. Gene set enrichment analysis (GSEA) demonstrates that NUP98-PHF23 fusion shares gene expression signature of NUP98-HOXA9 fusion, the prototype of the NUP98-associated fusions, as well as the signature of leukemic stem cells. To our knowledge this is the first transcriptome analysis of human samples with NUP98-PHF23 positive AML. Our findings are in support of the gene expression study of NUP98-PHF23 mouse model and validate the usefulness of the mouse model in developing therapeutic strategies for the treatment of subsets of AML.

1. Introduction

AML is a heterogeneous disease caused by genetic aberrations, epigenetic modifications, and deregulated expression of genes, leading to increased proliferation and decreased differentiation of hematopoietic progenitor cells. Chromosome rearrangements, including balanced translocations, are frequent findings in AML leading to the expression of chimeric fusion proteins [1]. Rearrangements involving NUP98 have been reported in a wide array of hematopoietic malignancies [2,3]. Due to its participation in the formation of fusion oncoproteins with at least 29 different genes, NUP98 is considered as one of the most promiscuous fusion partner genes.

The NUP98 gene encodes a protein that is a component of the nuclear pore complex and contains multiple Gly-Leu-Phe-Gly (GLFG) repeats that are thought to function as docking sites to allow bidirectional transport of mRNA and proteins between the nucleus and the cytoplasm [4]. All the NUP98 translocations resulted in fusion of the 5′ region of NUP98 to the 3′ region of a partner gene. The partners can be categorized into homeodomain (HD) family and non-HD family [2]. A recurring theme among some of the non-HD fusion partners is the presence of a histone "reading" (recognition) or "writing" (application) domain. In NUP98-PHF23 fusion, the PHD domain of PFH23 is retained in the fusion [5,6]. It binds to H3K4me3 and functions as a putative aberrant chromatin modifier [7].

Although NUP98 fusions were initially considered to be infrequent, application of fluorescence in situ hybridization (FISH) and other molecular tools has revealed cryptic rearrangements disrupting the NUP98. In fact, in one study with application of RT-PCR, a single NUP98 fusion (NUP98-NSD1) was detected in 16.1% and 2.3% of pediatric and adult AML with apparently normal karyotype, respectively [8]. Moreover, NUP98 fusions have been found to be associated with an unfavorable clinical outcome [8–11].

In a previous study, we described the identification of a cryptic t(11;17) leading to a NUP98-PHF23 fusion in an adult patient with AML [5]. More recently, we interrogated the terminal region of chromosome 17p in a pediatric patient with AML and report that NUP98-PHF23 is a recurrent translocation in AML. A new report by Togni et al. detected NUP98-PHF23 fusion in two additional pediatric patients with AML [12]. Gough et al. demonstrated potent

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leukemogenic role of NUP98-PHF23 in a mouse model [13]. However, no gene expression analysis has been conducted in patients with NUP98-PHF23 positive AML. Taking the RNA sequencing approach, we characterized the t(11;17) translocation in our two patients and identified genes with consistent differential expression profile in both patients. We report here that NUP98-PHF23 fusion shares gene expression signatures of NUP98-HOXA9 fusion and leukemic stem cells.

2. Materials and methods

2.1. Patient samples

Bone marrow samples from the two patients described in this study were received in our laboratory for diagnostic purpose. Standard procedures for evaluating hematologic malignancies were performed, including morphology examination, flow cytometry analysis and karyotype analysis. Normal control samples used for gene expression analysis were bone marrow samples from transplantation donors. All of the samples used in this study were existing clinical samples in accordance with IRB approved protocols.

2.2. Karyotype and FISH analysis

Conventional karyotype analysis was performed on the two AML diagnostic samples for the detection of chromosome abnormalities. FISH was performed to characterize regions in question following initial karyotype analysis. A dual color break-apart rearrangement probe set containing bacterial artificial chromosome (BAC) clones RP11-120E20 and RP11-438N5 (BACPAC Resources, Oakland, CA, USA) was used to detect NUP98 rearrangement, as described previously [5].

2.3. RNA sequencing

Total RNA was isolated from bone marrow samples using QIAGEN RNeasy Mini Kit, and the Low Input RiboMinus Eukaryote System v2 (Life technologies, Frederick, MD, USA) was used to remove rRNA from 500 ng of total RNA. cDNA libraries were then constructed using Agilent SureSelect Strand-Specific RNA Library Prep Kit according to the manufactural protocol. Quantification was performed using Agilent 2100 Bioanalyzer. Each paired-end indexed library was sequenced to a length of 100 nucleotides per mate (2×100) at a depth of $\sim 30 \times 106$ mate-pairs using Illimina HiSeq 2000 instrument.

To detect the NUP98-PHF23 fusion transcripts in the two patients, we used a method described by Trapnell et al. [14]. Briefly, TopHat 2.0.20 was used to align the RNA-seq reads to hg19 genome, and Cufflinks v2.1.1 was used to assemble transcripts, estimates their abundances, and tests for differential expression and regulation. The alignments from TopHat were used as input and default parameters were used.

To characterize differential expression of genes, we imported the raw FASTQ files generated from the RNA-seq into CLC Genomics Workbench 7.0.2. The transcriptome analyses were carried out using default settings on the Workbench and per million mapped reads (RPKM) values were log2 transformed prior to comparison of NUP98-PHF23 positive AML vs. normal controls.

2.4. Gene set enrichment analysis

Gene set enrichment analysis (GSEA) is a computational method that determines whether a priori defined set of genes shows statistically significant and concordant differences between two biological states, such as normal vs. malignancy [15,16]. GSEA studies of RNA-seq data from three normal controls and two available NUP98-PHF23 positive AML patients were carried out using the Broad Institute program (http://www.broadinstitute.org/gsea/index.jsp).

3. Results

3.1. NUP98-PHF23 fusion is a recurrent event in AML

Translocation t(11;17) resulted in chromosome breaks in the terminal bands of 11p and 17p, respectively. This translocation appeared to be a cryptic event that is difficult to detect by conventional karyotyping. Similar to what we observed for patient 1 [5], this translocation was first questioned for a slightly different banding pattern in the terminal region of patient 2. Initial karyotype of a bone marrow specimen from patient 2 detected three copies of chromosome 3 (trisomy 3) as well as a questionable terminal band of 17p. Using a commercially available FISH probe targeting the terminal band of 17p, we observed a FISH signal pattern consistent with a balanced translocation between 11p and 17p. Our previous finding of NUP98-PHF23 fusion in patient 1 prompted us to investigate the possibility of a recurrent translocation in patient 2. Using the NUP98 and PHF23 FISH probes developed in our previous study, we confirmed that t(11;17) disrupting NUP98 is indeed a recurrent translocation in AML (Fig. 1). This is the fourth reported AML case of t(11;17) with NUP98-PHF23 fusion.

3.2. Determination of NUP98-PHF23 fusion point

Using RNA sequencing, we determined the NUP98-PHF23 fusion points in both patients. For NUP98, both patients had the same fusion point at the end of exon 13 (NM_016320.4, nt. 1963). For PHF23, the fusion point in patient 1 was within exon 4, which has been further confirmed by Sanger sequencing following long-rang PCR and cloning [17]. This fusion used a known, alternate splice acceptor within PHF23 exon 4 (isoform 2; NM_001284517.1). Patient 2 used exon 4 splice acceptor in PHF23 isoform 1 (NM_024297.2), which is identical to the two newly reported NUP98-PHF23 positive AML [12]. Each case resulted in an in-frame NUP98-PHF23 fusion (Fig. 2). The complete sequence of the fusion product has GenBank accession number EF071958 for patient 1, and KU847959 for patient 2.

RNA sequencing also detected a reciprocal PHF23-NUP98 fusion transcript in patient 2, between PHF23 exon 4 and NUP98 exon 13. However, no reciprocal fusion transcript was evident in patient 1, who used an alternate splice acceptor within PHF23 exon 4.

3.3. Features of NUP98-PHF23 fusion

Both of our patients received the diagnosis of AML, and their clinical features are summarized in Table 1. Both patients achieved clinical and cytogenetic remission following treatment, but relapsed in 11 and 7 months, respectively. At the time of diagnosis the pediatric patient (patient 2) was treated with COG-AALL1031 (https://www.childrensoncologygroup.org/index.php/aaml1031), and had re-induction on POETIC study (Plerexifore, high dose Ara-C and Etoposide with intrathecal Cytarabine) at the time of relapse. The course was complicated by prolonged fevers, Ara-C syndrome, and dysfunctional uterine bleeding. Consistent with other reported AML cases involving NUP98 fusions, both patients experienced an aggressive disease course and ultimately succumbed to death.