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The seventh pathogenic fusion gene *FIP1L1-RARA* was isolated from a t(4;17)-positive acute promyelocytic leukemia

The majority of acute promyelocytic leukemia (APL) cases are characterized by the expression of the chimeric fusion gene *PML-RARA*. Although the *PML-RARA* fusion gene is detected in more than 95% of APL cases, *RARA* has also been found to fuse with other partner genes in some APL variants. To date, five such partner genes have been reported: *PLZF*, *NPM*, *NuMA*, *Stat5b* and *PRKAR1A*.[1,2] These fusion gene products however, must meet a number of common prerequisites for APL pathogenesis to ensue. The *RARA* gene portion of the fusion gene products ought to be from exon 3, and the fusion gene products must form homodimers as well as repress retinoic acid-responsive transcriptional activity.[3,4] We hereby report the cloning of a seventh fusion gene from an APL variant and the functional characterization of its product.

A 90 year-old woman was clinically diagnosed for APL. The karyotype was 47, XX, t(4;17)(q12;q21), +8. FISH analysis showed that 94% of the bone marrow cells had the *RARA* split signal without the *PML-RARA* fusion signal (Figure 1A).

To identify the 5'-fusion partner of *RARA*, we adopted the 5'-RACE method (5'-Full RACE Core Set, Takara Bio) according to the manufacturer's instructions. Briefly, the reverse primer 5'-GCGCTTTGCGCACCT-3' was designed, which was complimentary for exon 3 of the *RARA* gene. Following reverse transcription using total mRNA from the patient's bone marrow cells, the cDNA obtained was ligated by T4 RNA ligase. The ligated product was amplified by the nested polymerase chain reaction (PCR). PCR primer sequences were as follows: 1st PCR primers (5'-CTGCAGAAGTGCTTTGAAGT-3', 5'-CACCTTGTTGATGATGCAGT-3') and 2nd PCR primers (5'-GAGTGCTCTGAGAGCTACAC-3', 5'-CGGTGACACGTGTACACCAT-3'). The products obtained were cloned and sequenced directly. As a result, *FIP1L1* was identified as the fusion partner of *RARA* (Figure 1B). The *RARA* portion in this case starts, as expected, from exon 3 and is fused to exon 15 of *FIP1L1*. While cloning the full length *FIP1L1-RARA*, we isolated three isoforms of *FIP1L1-RARA*; all of these isoforms are in-frame (Figure 1C). We also confirmed the mRNA expression of *RARA-FIP1L1* by means of RT-PCR analysis (data not shown). *FIP1L1* is known to form a fusion gene with *PDGFRA* that causes chronic eosinophilic leukemia.[5] In a similar fashion to *FIP1L1-PDGFRA*, which produces several isoforms caused by alternative splicing, the isoforms of FIP1L1-RARA also seemed to be generated by alternative splicing of the *FIP1L1* portion.[6] *FIP1L1-RARA* was recently isolated from a case of juvenile myelomonocytic leukemia (JMML).[7] In the JMML case, as in our case, the fusion gene was generated between exon 15 of *FIP1L1* and exon 3 of *RARA*. At the moment, the reason why FIP1L1-RARA causes two different phenotypes of leukemia is unknown, nevertheless we propose two hypotheses. One possibility is that the difference in cell lineage derived from the identical fusion gene may be due to some additional mutation, allowing for the progression of a particular disease and not another. Alternatively, the fusion gene may target different progenitor populations and influence the phenotype.

FIP1L1-RARA has already been isolated; however, the function of the gene product has not yet been analyzed. Thus, we examined the potential of FIP1L1-RARA to form a homodimer. The full length cDNAs of *FIP1L1*, *RARA* and *FIP1L1-RARA* were cloned into the T7-tagged

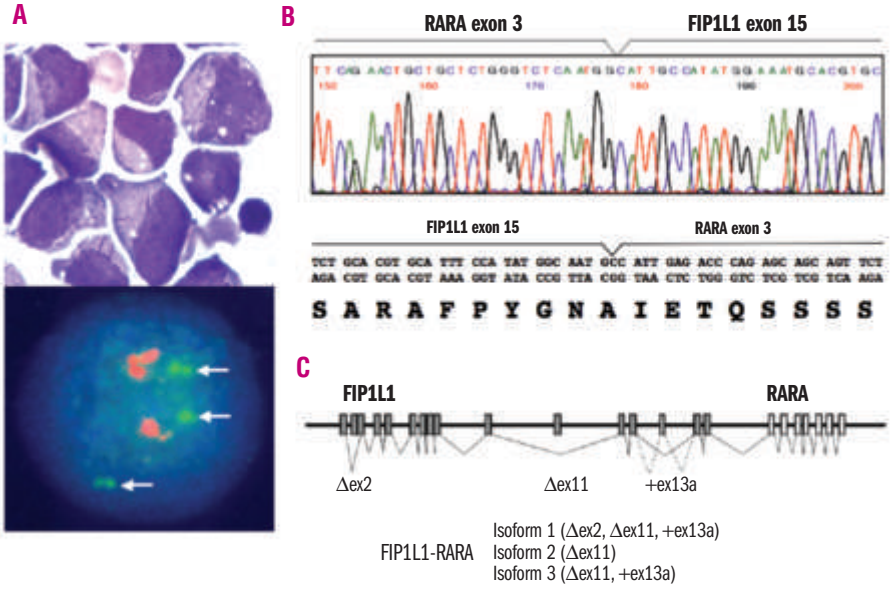

Figure 1. ***FIP1L1-RARA*** **was identified from t(4;17)-positive APL cells. (A) Morphology of the leukemia cells shows hypergranular promyelocytes with Auer rods (upper panel). FISH, using a *PML* probe (red signal) and a *RARA* probe (green signal), was performed for nuclei of a leukemia cell in interphase. Split FISH signals of *RARA* (arrow) indicate rearrangement of *RARA* (lower panel). (B) The sequence analysis of the identified fusion gene from the reverse sequence of *RARA* exon 3 identified *FIP1L1* as the fusion partner gene. The fusion gene between *FIP1L1* and *RARA* is in frame and the translated amino acid sequence is shown. (C) Schematic representation of the estimated organization of *FIP1L1-RARA* rearrangement at the genomic level and the isolated isoforms. Isoform 1 lacks exons 2 and 11 and gains an additional exon 13a. Isoform 2 lacks exon 11. Isoform 3 lacks exon 11 and gains exon 13a.**

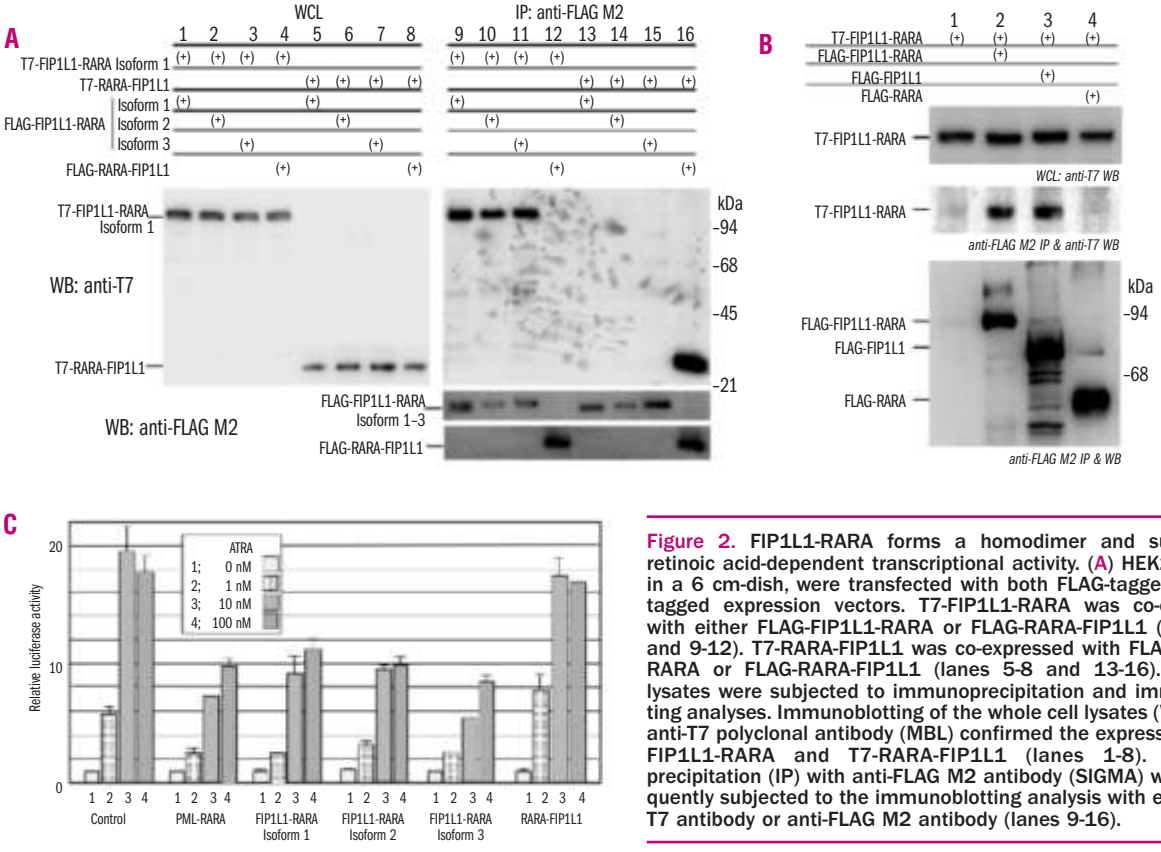

Figure 2. FIP1L1-RARA forms a homodimer and suppresses retinoic acid-dependent transcriptional activity. (A) HEK293 cells, in a 6 cm-dish, were transfected with both FLAG-tagged and T7-tagged expression vectors. T7-FIP1L1-RARA was co-expressed with either FLAG-FIP1L1-RARA or FLAG-RARA-FIP1L1 (lanes 1-4 and 9-12). T7-RARA-FIP1L1 was co-expressed with FLAG-FIP1L1-RARA or FLAG-RARA-FIP1L1 (lanes 5-8 and 13-16). The cell lysates were subjected to immunoprecipitation and immunoblotting analyses. Immunoblotting of the whole cell lysates (WCL) with anti-T7 polyclonal antibody (MBL) confirmed the expression of T7-FIP1L1-RARA and T7-RARA-FIP1L1 (lanes 1-8). Immunoprecipitation (IP) with anti-FLAG M2 antibody (SIGMA) was subsequently subjected to the immunoblotting analysis with either anti-T7 antibody or anti-FLAG M2 antibody (lanes 9-16). (B) T7-FIP1L1-RARA was co-expressed with mock (lane 1), FLAG-FIP1L1-RARA (lane 2), FLAG-FIP1L1 (lane 3) or FLAG-RARA (lane 4). Immunoblotting of the WCL with anti-T7 antibody confirmed the expression of T7-FIP1L1-RARA (upper panel). IP with anti-FLAG M2 antibody was subsequently subjected to immunoblotting analysis with either anti-T7 antibody (middle panel) or anti-FLAG M2 antibody (lower panel). (C) HEK293 cells, in 35 mm-dishes, were transfected with 0.25 μg of the retinoic acid responsive-luciferase vector, which contains seven repeats of the retinoic acid-response element (RARE) in the *RARβ2* gene, combined with 2 μg of empty vector or the expression vectors FLAG-PML-RARA, FLAG-FIP1L1-RARA or FLAG-RARA-FIP1L1 respectively. One day after transfection, the culture media were exchanged with fresh culture media supplemented with the indicated concentration of ATRA. Following two days of ATRA treatment, the cells were harvested and luciferase activities were analyzed. The luciferase activity without ATRA treatment was arbitrarily assigned as 1.0 and the results are shown as the mean ± SD. The analysis was performed in triplicate assays and the results were reproducible.

or FLAG-tagged expression vectors, and these vectors were transiently transfected and used for immunoprecipitation and immunoblotting analysis. The result revealed that all three identified isoforms of FIP1L1-RARA (Figure 2A, lanes 9-11), as well as RARA-FIP1L1 (Figure 2A, lane 16), form homodimers. Also, FIP1L1-RARA associated with either FIP1L1-RARA or FIP1L1, but not with RARA (Figure 2B), confirming that the homodimerization of FIP1L1-RARA is dependent on the FIP1L1 portion. Interestingly, the FIP1L1 portion of FIP1L1-PDGFRA does not have the ability to form a homodimer.[8] The breakpoint in *FIP1L1-RARA* is in intron 15 of *FIP1L1*. On the other hand, the breakpoints in *FIP1L1-PDGFRA* were distributed among introns 10 to 13 of *FIP1L1*,[9] which leads us to believe that the different breakpoints of *FIP1L1* in *FIP1L1-RARA* and *FIP1L1-PDGFRA* may confer different attributes upon the fusion products at the point of homodimerization.

Subsequently, we examined the effect of FIP1L1-RARA on the retinoic acid response. FIP1L1-RARA represses retinoic acid-dependent transcriptional activity at the lowest concentration of all-trans retinoic acid (ATRA), as determined by the luciferase assay (Figure 2C). At higher concentrations of ATRA, luciferase activity is induced. As shown in Figure 2C, FIP1L1-RARA has an ATRA response similar to that of PML-RARA. Consistent with this result, our patient achieved a complete remission by oral administration of ATRA alone (50 mg daily) (Mori *et al.*, manuscript in preparation).

In our examination, the expression of the reciprocal *RARA-FIP1L1* fusion gene was also detected. The analysis of PML-RARA transgenic mice suggested that the reciprocal product, a particular isoform of RARA-PML transcripts, plays a role in disease progression.[10,11] At this moment we are not suggesting that RARA-FIP1L1 has any functional role in leukemogenesis, but the homodimerization of RARA-FIP1L1 implies that it may possess a pathological function.

To summarize, FIP1L1-RARA forms a homodimer and represses the retinoic acid response. We therefore propose that *FIP1L1-RARA* is the seventh pathogenic fusion gene of APL.

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Early discharge from hospital after consolidation chemotherapy in acute myeloid leukemia in remission: febrile neutropenic episodes and their outcome in a resource poor setting

Treatment of acute myeloid leukemia (AML) involves administration of myelosuppressive chemotherapy administered after admittance to hospital.[1] Admission to intensive nursing care units till bone marrow recovery leads to prolonged hospital stay. Quality of life and health care issues have made many cancer centers change to outpatient care even during high-risk phases of disease.[2-6]

In India most patients belong to poor socioeconomic backgrounds. There is an acute shortage of hospital beds. Early discharge after myelosuppressive therapy would promote better use of hospital resources, but the safety of this approach in these patients has not been established. We present our experience of the feasibility and safety of early discharge of patients with acute myeloid leukemia after consolidation chemotherapy.

All patients were induced with standard '3+7' chemotherapy using a peripherally inserted central venous (PICC) line. After documentation of complete remission (CR) consolidation chemotherapy with 3 cycles of high dose cytarabine was given.

Eighty-three consecutive episodes of neutropenia after consolidation chemotherapy in 28 acute myeloid leukemia patients in remission were included in the study. Patients were divided into 2 groups.

Group 1. Outpatients. These consisted of patients discharged after the chemotherapy was completed to their own homes or temporary residential facilities, which did not have any medical, or home visit facilities. Criteria for inclusion were: (a) no fever or infection; (b) location of residence nearby; (c) ability to come to hospital within one hour if fever developed or condition deteriorated. They had telephone access to the study team.

Group 2. Inpatients. These were patients who remained in hospital after high dose Ara C (HiDAC) chemotherapy till recovery of neutrophil counts. The criteria of inclusion were: (a) inability to move to a residential place as specified under group 1 or (b) severe infective course during earlier chemotherapy.

All patients were given the following supportive therapy: prophylactic ciprofloxacin 500 mg twice daily and fluconazole 200 mg/day and simple instructions concerning hygiene. Blood counts were monitored twice a week for Outpatients and on alternate days for Inpatients. Outpatients were seen at least once a week in the outpatient department (OPD). Blood and platelet transfusion support was given in the day care center. Patients who developed fever were administered granulocyte colony stimulating factor (G-CSF). Fever was considered present if the temperature measured orally was ≥38° C on two occasions at least four hours apart during a 24-hour period or was ≥38.5°C on a single occasion. Neutropenia was defined as an absolute neutrophil count (ANC) of $<0.5\times10^9$/L.[7]

All Outpatients who developed fever were admitted and administered IV antibiotics. First line antibiotics included piperacillin-tazobactum or cefoperazone-sulbactum, along with amikacin. Second line empirical antibiotics were generally started if fever persisted for 48-72 hours and there was no clinical improvement. These consisted of a carbapenem or aztreonam. Vancomycin/teicoplanin were added at onset or later as per febrile neutropenia guidelines.[7] Amphotericin was added if there was any suspicion of fungal infection based on clinical or X-ray findings, and empirically if fever and neutropenia persisted despite antibiotic therapy for more than five days.

After resolution of fever and if there was no obvious infection, IV antibiotics were changed to oral antibiotics that were continued for at least five days or till ANC recovered. Outpatients were discharged once oral antibiotics (amoxycillin-clauvulanic acid and levofloxacin) were initiated, while Inpatients remained in hospital till recovery of ANC.

The number of febrile neutropenic episodes, use of antibiotics, patterns of infection and mortality after completion of HiDAC were compared in the 2 groups. The SPSS statistical software (Chicago, IL, USA) was used for analysis.