# Point Mutations in the *AML1/RUNX1* Gene Associated with Myelodysplastic Syndrome (MDS)

Hironori Harada,[1] Yuka Harada,[2] Akiro Kimura[1]

[1] Department of Hematology/Oncology, Research Institute for Radiation Biology and Medicine, Hiroshima University, Hiroshima, Japan

[2] International Radiation Information Center, Research Institute for Radiation Biology and Medicine, Hiroshima University, Hiroshima, Japan

Myelodysplastic syndrome (MDS) is a clonal disorder of hematopoietic stem cells characterized by ineffective and inadequate hematopoiesis. MDS in a subset of patients arise after previous chemotherapy or radiation exposure for other malignancies. As MDS is a heterogeneous disorder, specific gene abnormalities playing a role in the myelodysplastic process have been difficult to identify. In this study, we analyzed the somatic mutations in the *AML1/RUNX1* gene, which is a critical regulator of definitive hematopoiesis and the most frequent targets for translocation of acute myeloid leukemia (AML), in patients with MDS. We detected AML1 point mutations in 26 of 110 (23.6%) patients with refractory anemia with excess blasts (RAEB), RAEB in transformation (RAEBt) and AML following MDS (defined these categories as MDS/AML). Among 22 patients with radiation-related (including 14 atomic bomb survivors) and/or therapy-related MDS/AML, 11 (50%) patients had the AML1 mutations mostly in N-terminal region. In contrast, 15 of 88 (17%) patients with sporadic MDS/AML showed the AML1 mutations equally in both N-terminal and C-terminal region. The MDS/AML patients with AML1 mutations had a significantly worse prognosis than those without AML1 mutations. Most of AML1 mutants lost *trans*-activation potential, regardless of their DNA binding potential. These data suggested that AML1 point mutation is one of the major driving forces of MDS/AML, and these mutations may represent a distinct clinicopathologic-genetic entity.

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## Introduction

The myelodysplastic syndrome (MDS) is an umbrella term for a heterogeneous collection of hematopoietic stem cell disorders affecting elder adults. MDS is a distinct group of clonal hematopoietic disorders characterized by ineffective hematopoiesis, refractory cytopenias and a tendency to progress into acute myeloid leukemia (AML). Most of patients found to have MDS are older than 60 years of age. A substantial proportion of MDS arise in the setting of exposures to radiation including atomic bomb, occupational toxins or cytotoxic therapy for a prior malignancy. The French-American-British (FAB) cooperative group proposed 5 subgroups of MDS based mainly on the percentage of leukemic blasts in the bone marrow (BM) and peripheral blood (PB).[1] That is, refractory anemia (RA), RA with ringed sideroblasts (RARS), chronic myelomonocytic leukemia (CMML), RA with excess blasts (RAEB, 5% to 20% blasts in BM), and RAEB in transformation (RAEBt, 20% to 30% blasts in BM). Recently, the World Health Organization (WHO) proposed a classification for MDS,[2] but its clinical and pathological relevance has been thrown into question, compared with its classification for leukemias based on their cytogenetic and genetic abnormalities.[3]

Genes encoding transcriptional factors that play critical roles in hematopoiesis are frequently involved in the genetic alterations in leukemia and MDS. For example, the *AML1/RUNX1* gene was initially identified as a gene encoded on chromosome 21 in the breakpoint of the t(8;21) AML.[4] AML1 is a family member of runt-related transcriptional factors. It recognizes the consensus DNA sequence TGT/cGGT, through its runt homology domain (RHD) located near its amino (N)-terminus, and its affinity for DNA increases by heterodimerization through the RHD with a ß subunit (CBFß). Transcription activation domains and transcription repression domains are located in carboxy (C)-terminal region. AML1 alone has weak intrinsic transactivation potential, but synergistically activated transcription is seen by forming a complex with other transcriptional factors which has adjacent binding sites and/or coactivators. AML1-CBFß complex plays an important role for establishment of all lineages of
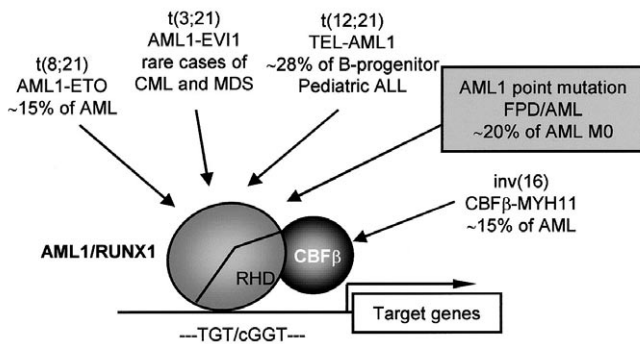
**Figure 1.** AML1/CBFß transcription factor complex and hematological malignancy.



**Figure 2.** Methods for detection of the AML1 point mutations.

definitive hematopoiesis, which has been demonstrated by the analysis of *AML1*- and *CBFß*- deficient mice.[5-9] AML1/CBFß transcriptional factor complex is also well known as one of the most frequent targets of chromosomal translocations (Figure 1). *AML1* has been identified as a partner gene in several leukemia-associated chromosomal translocations, resulting in the formation of chimeric oncoproteins.[10] In addition to chromosomal translocations, heterozygous germline mutation of *AML1* gene is also known to be causative for familial platelet disorder with predisposition to AML (FPD/AML),[11-13] in which haploinsufficiency of AML1/RUNX1 causes an autosomal dominant disorder characterized by congenital platelet defects and propensity to develop AML with high incidence (20-50%). Moreover, AML1 mutations have been detected in about 20% cases of poorly differentiated AML M0 subtype.[14-17]

The *AML1* gene also was reported as a target of gene alteration by ionizing radiation and anticancer drugs in experimental systems.[18,19] Moreover, human leukemias associated with *AML1* gene translocations after anticancer therapy or low-dose radiation have been reported.[20,21] These data prompted us to test the frequency of point mutations in the *AML1* gene in patients with hematological malignancies, including atomic bomb survivors in Hiroshima. We found a high frequency of *AML1* mutations in patients with RAEB, RAEBt and AML following MDS (defined these 3 disease categories as MDS/AML),[22] especially among radiation-associated and therapy-related MDS/AML.[23] Our data suggest that AML1 point mutations are strongly associated with a specific type of hematopoietic malignancy, and radiation and anticancer drugs contribute to the development of MDS/AML through mutations of the *AML1* gene.

## Materials and Methods

We examined a total of 377 cases of hematological diseases including MDS, AML, chronic myeloid leukemia (CML), myeloproliferative disorder (MPD), and acute lymphoid leukemia (ALL) (see reference 22 for details). Diagnosis was based on morphologic and immunophenotypic studies according to the FAB classification. Fourteen cases of atomic bomb survivors in Hiroshima who exposed atomic bomb within 3 km of the hypocenter were included in the
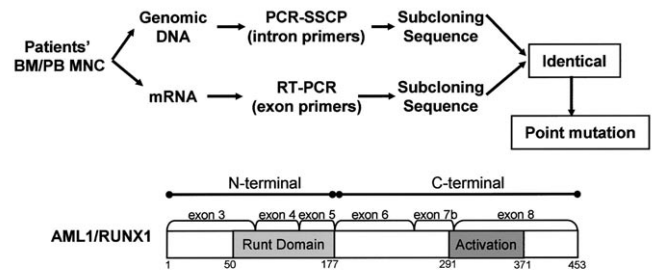
patients with MDS/AML. Patient samples were taken after obtaining informed consent and approval from the institutional review board at Hiroshima University. Mononuclear cells (MNCs) were isolated from bone marrow (BM) or peripheral blood samples, and genomic DNA or total RNA was extracted. Polymerase chain reaction-single-strand conformation polymorphism (PCR-SSCP) of exons 3 through 8 was performed using the flanking intronic primers as described.[22,23] To confirm the mutations, PCR products from cDNA were also sequenced. The methods for identification of *AML1* mutations were summarized in Figure 2. Functional analysis (DNA-binding ability, CBFß-binding ability and trans-activation potential) on AML1 mutants were performed as described elsewhere.[22,23]

## Results

### High frequency of AML1 mutations in MDS

To investigate the AML1 mutations in hematological diseases, we analyzed exons 3 through 8 of the *AML1* gene. Point mutations were detected in patients with MDS and AML, whereas no mutation was detected in the patients with CML, MPD and ALL (Table 1). Twenty-six of 110 patients (23.6%) with RAEB, RAEBt and AML following MDS had AML1 mutations, and 17 mutations located in the N-terminal and 9 in the C-terminal. N-terminal AML1

**Table 1.** Frequency of AML1 mutations in hematological diseases

| Diagnosis | AML1 mutation | | |
|---|---|---|---|
| | N-terminal | C-terminal | Total |
| RAEB, RAEBt, MDS AML (MDS/AML) | 17 | 9 | 26/110 (23.6%) |
| RA, RARS | 1 | 0 | 1/46 (2.2%) |
| CMML | 0 | 0 | 0/4 |
| de novo AML | 5 | 0 | 5/115 (4.3%) |
| CML | 0 | 0 | 0/23 |
| MPD | 0 | 0 | 0/51 |
| ALL | 0 | 0 | 0/28 |
| Total | 23 | 9 | 32/377 |

mutations were also found in 1 of 46 patients with RA/RARS and 5 of 115 patients with AML without antecedent MDS, but no C-terminal AML1 mutations were found in those patients. Thus, we found a strong correlation between AML1 mutations (especially those in the C-terminal region) and the subgroups of MDS; i.e., RAEB, RAEBt and AML following MDS (defined these 3 disease categories as MDS/AML).

To investigate the AML1 mutations in radiation-associated and therapy-related MDS/AML, all the patients with MDS/AML were traced to previous exposure history. Twenty-two patients who had histories of radiation exposure and/or chemotherapy, including 14 atomic-bomb survivors, were defined as "secondary" MDS/AML. AML1 mutations were identified in 11 (50%) patients with secondary MDS/AML, and all but one patient had the N-terminal mutations (Figure 3). By contrast, 15 of 88 (17%) patients with sporadic MDS/AML showed AML1 mutations, half of them occurring in the C-terminal region.

To test whether AML1 mutations affect the prognosis of MDS/AML, we tracked the overall survival of patients with MDS/AML, comparing survival of those associated with AML1 mutations (n=26) with that of patients without the mutations (n=81) (Figure 4). AML1 mutations were proven to be a risk factor by log-rank test.
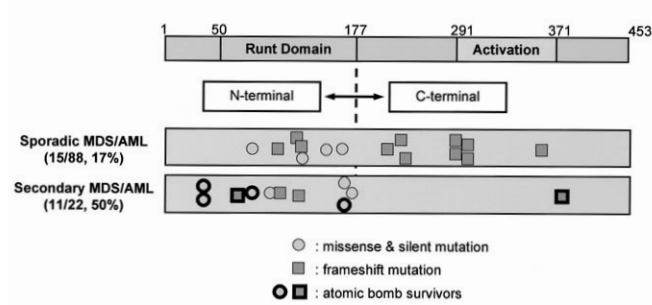
*Functional analysis of AML1 mutants*

In the N-terminal region, we found both missense/silent mutations and frame shift mutations that resulted in truncation of the authentic protein, whereas all of the C-terminal mutations resulted in frame shifts, of which the consequences were unusual. Usually, frame-shift mutations result in truncation of the authentic protein followed by a relatively short additional stretch of amino acid residues originating from the wrong reading frame or from intronic sequences. But in 4 cases of C-terminal mutations, the stretches of additional amino acids resulting from the wrong reading frame were even longer than wild-type, because the wrong frame contained an in-frame termination codon 353 bp downstream of the authentic termination codon. Thus, the mutated AML1 proteins in these 4 cases appeared to be fusion proteins rather than truncations of AML1.

To obtain insight into molecular mechanisms through which the AML1 mutations contribute to malignant transformation of hematopoietic progenitors, we analyzed the biochemical functions including DNA-binding ability, CBFß-binding ability and *trans*-activation potential of AML1 mutants. The results of the functional analysis of each AML1 mutants were compared with wild-type AML1 and were summarized in Figure 5. The AML1 mutants were divided into N-
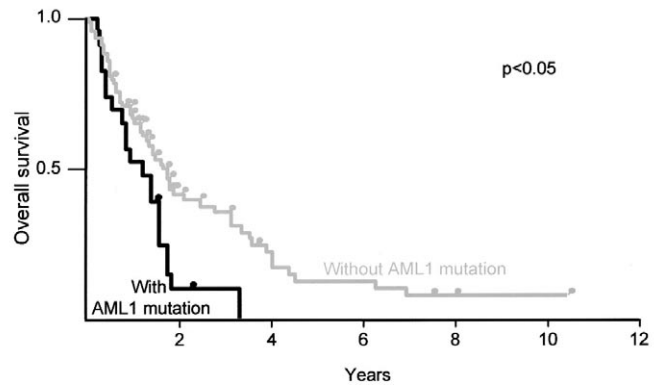


**Figure 3.** AML1 point mutations identified in MDS/AML.



**Figure 4.** Kaplan-Meier analysis comparing overall survival of patients with MDS/AML: with AML1 point mutation vs. without AML1 mutation.
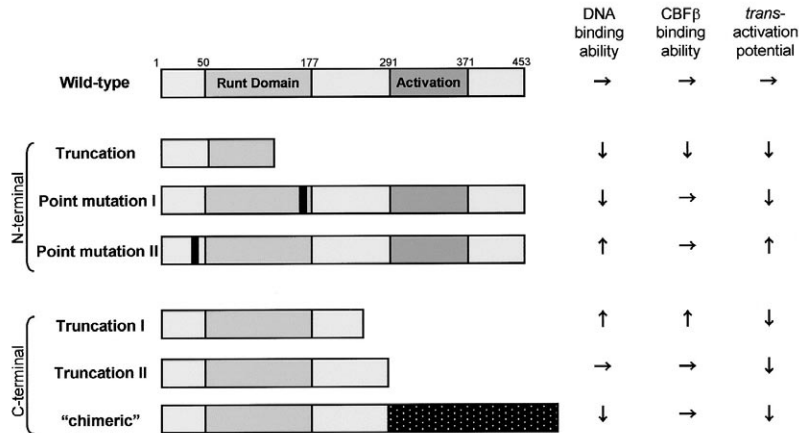


**Figure 5.** Biochemical functions of the AML1 mutants.

terminal type and C-terminal type. Most of N-terminal mutations result in alteration of RHD. Thus, these mutants are predicted to lose DNA-binding ability and *trans*-activating potential. C-terminal mutants with intact RHD provided 2 additional types of mutation. Truncation type of mutants has normal or enhanced DNA-binding ability but no *trans*-activation potential. Chimeric type of mutants, which is longer than wild-type AML1 and appears to be fusion proteins, has attenuated DNA binding potential without *trans*-activation potential. In spite of such divergent biochemical features, it is suggested that AML1 mutants lose their *trans*-activation potential and contribute equally to the same type of myeloid malignancy, MDS/AML.

## Discussion

In this study, we established strong correlations between point mutations of the *AML1* gene and subgroups of MDS, i.e., RAEB, RAEBt, and AML following MDS. We define these 3 disease categories as MDS/AML. Of the 110 patients with MDS/AML who were tested, 26 (23.6%) had an AML1 mutation. Conversely, of 32 patients with AML1 point mutation, 26 (81%) belonged to this category (Table 1). Moreover, the prognosis for the patients with AML1 mutations was significantly worse than for patients without AML1 mutations (Figure 4). The MDS/AML category is characterized by (1) relatively a low blast percentage in the bone marrow, (2) multilineage dysplasia, and (3) poor prognosis. Moreover, *AML1* is the first gene that was demonstrated to have a high frequency of mutations in patients with MDS/AML but rare in patients with other types of hematologic diseases. Thus, 'MDS/AML with AML1 mutation' may represent a new subgroup, which would be considered for inclusion in the recurrent genetic abnormalities in the WHO classification. Our data suggested that AML1 mutation is one of the major driving forces of MDS/AML, and may represent a distinct clinicopathologic-genetic entity.

We also reported that AML1 point mutations were detected in virtually half of late-onset MDS/AML patients among the atomic bomb survivors of Hiroshima. It may be thought that hematological malignancies among the atomic bomb survivors are extremely special, but they are very informative to consider the mechanisms of development of leukemia and MDS. As shown in Figure 6, acute and chronic leukemias among atomic-bomb survivors appeared after a minimum latency period of 2-3 years, reached a maximum after 6-7 years, decreased slowly with time and then returned to the back ground level in 30 years.[24] The incidence of leukemias was sharply dose-dependent. In contrast, other tumors including MDS showed apparently different kinetics. The incidence of other tumors increased after long latency periods of 10 or more years, continued to increase with time and still high even now, more than 50 years after exposure. The dose-dependency of the risk of these diseases was less prominent. This apparent difference in the pattern of the onset between leukemia and MDS may be interpreted by molecular mechanisms that contribute to the transformation of hematopoietic progenitors. Leukemogenic
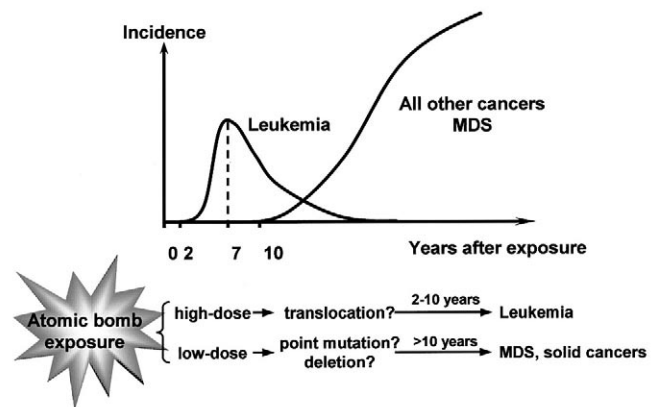


**Figure 6.** The difference in the pattern of the onset between leukemia and MDS among atomic bomb survivors.

fusion genes as a result of nonrandom chromosomal translocations are detected in approximately half of acute leukemia patients, whereas many of MDS are generally considered to develop as a result of the accumulation of gene deletions and point mutations.[25] Chromosomal translocations caused by double-strand DNA breaks resulting from high dose radiation are likely to contribute to the development of leukemia after a short latency time, whereas point mutations of genes induced by low-dose radiation may contribute to the development of MDS among atomic bomb survivors decades later. The reason why atomic bomb survivors have increased risks of various cancers even 50 years after a single radiation exposure is because radiation-induced mutations required for the initiation of carcinogenesis were presumably record in long-lived stem cells with self-renewal capacity in various organs.[26,27]

Loss of AML1 function caused by the *AML1* mutations in hematopoietic stem cells is not sufficient for an individual to develop MDS/AML, and this explains the long latency period before development of AML among people having FPD/AML pedigrees with congenital *AML1* mutations. This suggests that the acquisition of some additional genetic alterations that cooperate with the AML1 mutations is needed for development of MDS/AML. Figure 7 shows a stepwise genetic progression model of MDS/AML with AML1
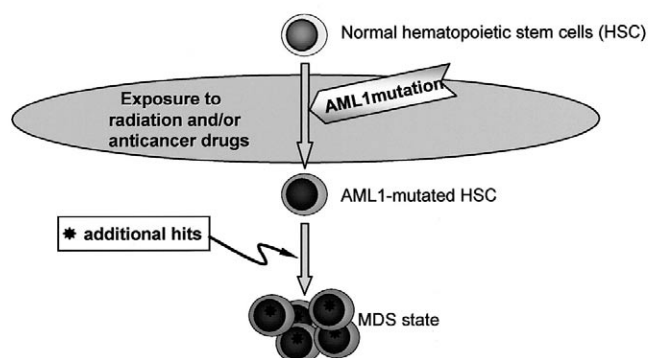


**Figure 7.** A stepwise genetic progression model in MDS/AML with AML1 point mutations by radiation and/or anticancer drugs.

point mutation. One hematopoietic stem cell that acquired *AML1* gene mutation by atomic bomb or other toxic reagents took decades to be transformed by accumulation of additional gene alterations leading to development of MDS/AML. To identify these additional genetic alterations cooperating with *AML1* mutations is important for clarifying the mechanism of development of MDS/AML.

## Acknowledgements

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