SETBP1 mutations in 106 patients with therapy-related myeloid neoplasms

Therapy-related myeloid neoplasms (t-MN) are myeloid disorders, including acute myeloid leukemia (AML) and myelodysplastic syndromes (MDS) developing in patients treated with radiotherapy and/or chemotherapy for cancer or autoimmune diseases. Cytotoxic therapy may induce chromosomal alteration and genetic mutations in hematopoietic progenitors leading to leukemogenesis in susceptible subjects.

t-MNs are characterized by high incidence of complex karyotypes and frequent monosomies and/or deletions of chromosomes 7 and/or 5,[1] whereas the recently identified mutations of epigenetic regulators and of the spliceosome machinery are rare, with the exception of SRSF2.[2,3]

Recently, new sequencing technologies have enabled large screening of somatic mutations in myeloid malignancies, leading to the discovery of new hot spot mutations in genes candidate for leukemic transformation. Among these, SET binding protein 1 (SETBP1) has been reported as frequently mutated in chronic myelomonocytic leukemia (CMML), atypical chronic myeloid leukemia (aCML), secondary acute myeloid leukemia (s-AML) and in distinct subgroups of primary myelodysplastic syndromes (MDS), such as refractory anemia with excess of blasts (RAEB1 and RAEB2).[4-10]

SETBP1 germ-line mutations are responsible for the Schinzel-Giedion syndrome (SGS), originally identified by Albert Schinzel and Andreas Giedion in 1978. It is a congenital disease characterized by a higher prevalence of tumors, severe mid-face hypoplasia, congenital heart defect and skeletal anomalies.[11] The *SETBP1* gene is localized on chromosome 18q21.1 and the missense mutations are predominantly located in the SKI-homologous region, mainly between codon 858 and 871.

Makishima *et al.* have previously shown a significant association between SETBP1 mutations and –7/del(7q) abnormalities (15 of 72; P=0.01) in a large cohort of 727 patients with myeloid malignancies.[4] SETBP1 mutations were more frequent in AML evolving from a previous MDS, and CMML patients (19 of 113 cases, 16.8%, and 22 of 152 cases, 14.5%, respectively), whereas they were less frequent in *de novo* AML (1 of 145 cases, <1%). Similarly, Fernandez-Mercado *et al.*, screening a population of 328 patients with myeloid disorders, found 14 SETBP1 mutations (4.3%), 7 of which in patients with -7/del(7q) (7 of 19, 36.8%).[7] Moreover, Hou *et al.* found 14 SEPBP1 mutations in a cohort of 430 (3.3%) MDS patients (FAB classification), in particular in association to monosomy 7 (20%), and a significantly higher incidence of concurrent ASXL1, EZH2 and SRSF2 mutations.[8]

To date, little is known regarding prevalence, clinical and prognostic role of SETBP1 mutations in t-MN. Since t-MN are characterized by high prevalence of chromosome 7 alterations and SRSF2 mutations, the aim of our study was to determine the frequency of SETBP1 mutations in a cohort of 106 patients affected by t-MN, diagnosed at our institution between January 1994 and September 2013. Patients had developed a t-MN at a median of six years (range 0.1-32 years) from treatment of the primary disease. Median bone marrow (BM) blasts were 18 (range 1-100). Karyotype was abnormal in 52 of 81 (64.2%) patients with available karyotype. Chromosome 7 alterations were present in 16 of 81 (19.7%) patients. Patients' main clinical characteristics are described in Table 1. The study was approved by our Institutional Review Board and was conducted according to good clinical and laboratory practice rules and the principles of the Declaration of Helsinki.

Table 1. Clinical characteristics of 106 patients with therapy-related myeloid neoplasm (t-MN).

	N. (range)
Patients	106
Median age (years, range)	69 (16-88)
Sex (M/F)	45/61
Type t-MN according to the WHO	
MDS	53
AML	53
Primary malignancy	
Lymphoproliferative disease	34
Breast cancer	27
Urogenital	17
Gastrointestinal	10
Thyroid	5
Lung	5
Other	8
Treatment for primary malignancy	
Chemotherapy	59
Radiotherapy	11
Chemiotherapy + radiotherapy	36
Karyotype	
Normal	29
Complex	22*
Del(7)	13
Other	17
Not evaluable	25

*Including Del(7) in 3 cases.

Mononuclear cells (MNCs) were separated from patients' BM at the time of initial diagnosis by Ficoll gradient centrifugation using Lympholyte-H (Cedarlane, Ontario, Canada). DNA was extracted using a QIAamp DNA Mini Kit (Qiagen Srl., Milan, Italy), following the manufacturer's instructions. Detection of SETBP1 mutations was performed by Sanger sequencing using the BigDye Terminator v.3.1 cycle sequencing kit (ABI PRISM 3100; Applied Biosystems/Life Technologies, Milan, Italy). According to Thol *et al.*, primer sequences were: Forward 5'-ACCAAAACCCAAAAGGGAAT-3', and reverse 5'-CGGTTTTGCAGGCTTTTC-3'.[9] t-MN patients had been tested for DNMT3A, IDH1, IDH2, SRSF2, U2AF1 and SF3B1 mutations.[2] All mutations were confirmed in independent experiments. Paired t-test was performed to test for association between mutations of *SETBP1* and other genes, and with patient karyotype.

Unexpectedly, frequency of SETBP1 point mutations in the SKI-homologous domain was very low in our t-MN patient cohort (3 of 106, 2.83%). The detected missense changes are predicted to have a damaging effect in the protein (PolyPhen2 tool). Two patients carried a G870S (COSM1234973) mutation, whereas there was one S869R mutation. The 2 SETBP1 G870S mutated patients were also mutated for the SRSF2 gene at position P95 (P95H and P95R with a contextual P96 insertion), whereas the carrier of S869R resulted wild type for SRSF2. Paired t-test showed a significant association (P=0.0332) between SETBP1 (3 of

106 patients, 2.83%) and SRSF2 (9 of 106 patients, 8.49%) mutations. This tight association has been previously reported by Hou *et al.* and Thol *et al.*[8,9] No other associations between SETBP1 mutations, spliceosome machinery (SF3B1 exons 13-16 and U2AF1) and epigenetic regulators (IDH1 R132, IDH2 R140 and R172 and DNMT3A R882) somatic mutations were found.

Looking at primary disease, each of the 3 mutated patients had been treated for a different type of primary tumor (non-Hodgkin lymphoma, breast and thyroid cancers), but interestingly, all patients had developed a therapy-related myelodysplastic syndrome (one RAEB1 and 2 RAEB2). None of the therapy-related AML patients resulted mutated. These results reflect the figures reported by Makishima *et al.* and Piazza *et al.* who described a low incidence of SETBP1 mutations in *de novo* AML and high frequencies in RAEB patients.[4,6]

In our t-MN patients, SETBP1 mutations were not associated to chromosome 7 abnormalities. In fact, the carriers of a G870S mutation had a complex karyotype without chromosome 7 aberrations, whereas the patient with a S869R mutation had a deletion of chromosome 7. The low number of SETBP1-mutated t-MN cases precluded any-survival analysis in this cohort.

In conclusion, testing the largest t-MN cohort reported to date (106 patients), we found a low incidence of SETBP1 mutations. We confirm the association between SETBP1 and SRSF2 mutation, whereas the previously reported association between SETBP1 mutation and chromosome 7 alterations was not present in t-MN patients, suggesting that this abnormality may not be involved in the pathogenesis of these diseases. The low frequency of SETBP1 mutation in t-MN, similar to most epigenetic and spliceosome mutations, may suggest that other major changes, such as complex and monosomal karyotypes, together with TP53 mutations, may play the major role in therapy-related leukemogenesis.

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