TP53 Mutations in Myeloid Malignancies are either Homozygous or Hemizygous due to Copy Number-Neutral Loss of Heterozygosity or Deletion of 17p

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Our previous studies demonstrated that single nucleotide polymorphism arrays (SNP-A), applied as a karyotyping platform, complement traditional metaphase cytogenetics (MC) and improve the diagnostic yield of cytogenetic studies because SNP-A can more precisely resolve smaller genetic defects and allow for detection of copy number-neutral loss of heterozygosity (CN-LOH), a defect frequently found in myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML). Like balanced translocations, CN-LOH represents a chromosomal abnormality that occurs without a concurrent change in gene copy number (CN). CN-LOH is an increasingly recognized chromosomal mechanism by which homozygous somatic mutations may be acquired during evolution of hematologic malignancies and can pinpoint the location of such gene(s); examples include *CEBPA*, *FLT3*, *WT1*, *RUNX1*, *JAK2*, and *NF1*1.

Deletions involving the short arm of chromosome 17, detectable by MC in MDS and AML, may be associated with a complex karyotype that includes -5/5q- and/or -7/7q-; such patients have a poor prognosis2. The well known tumor suppressor gene *TP53*, located at 17p13.1, is a likely candidate gene contributing to the disease phenotype following mutation of one or

both alleles. Mutations may be linked to loss of 17p by chromosomal or submicroscopic deletion LOH (mut/-) or may occur in the absence of 17p loss in either heterozygous (wt/mut), biallelic (mut1/mut2) or homozygous (mut1/mut1) forms through CN-LOH. Although mutations in *TP53* are frequent in solid tumors, the mutation frequency reported in hematologic malignancies is much lower2.

We have applied high-density Affymetrix 250K and 6.0 SNP-A to identify LOH of chromosome 17 in a large cohort of patients (N=379) with MDS, MDS/MPN and primary and secondary AML. We noted that advanced forms of MDS and AML often harbored areas of LOH (deletion and CN-LOH) involving the short arm of chromosome 17 (Supplemental Table1 and Table 2).

In total we identified 21 (5.5%) patients with LOH17p on chromosome 17. Deletions (-17/17p-) were found in 14 (3.7%) and somatic CN-LOH17p in 7 (1.8%) patients by SNP-A. We defined a commonly deleted region (CDR) on chromosome 17p (Figure 1A). The CDR at 17p13.1 (bp 6,828,482 to 8,075, 871; 1.25 Mb) contained *TP53*, among others genes, and was present in 12/14 patients with del(17p). In two patients del(17p) did not span the *TP53* locus, suggesting that other genes located in these regions, such as *CRK* or *HIC1*, may be involved in disease pathogenesis. Somatic CN-LOH17p was detected in 7 patients. The average size of CN-LOH17p detected by SNP-A was 12.1 ± 5.5 Mb. The somatic origin of CN-LOH17p was confirmed by analysis of a control cohort consisting of 1003 individuals which did not reveal any cases of CN-LOH involving 17p, suggesting that large subtelomeric CN-LOH17p represents an acquired lesion. In addition, SNP-A was performed on paired CD3+ cells for exemplary cases of del(17p) and CN-LOH17p to confirm the somatic nature of these lesions (Figure 1B).

Comparison of the clinical characteristic of 21 patients with LOH17p revealed that these patients shared similar features including a complex karyotype and a strong association between LOH17p and abnormalities of chromosomes 5 and/or 7. Also, the majority of the 21 patients with LOH17p by SNP-A had aggressive histomorphologic features (10 secondary AML, 2 primary AML, 1 t-AML, 1 RAEB-1, 3 RAEB-2, 3 RCMD-RS, 1 RCMD) and a poor prognosis with a median survival of only 6 months for patients with del(17p) and 3 months in case of patients with CN-LOH17p (Figure 1C).

The identification of several cases with overlapping CN-LOH17p led us to hypothesize that *TP53* mutations would be present in these patients. Overall, 19/21 instances of LOH17p included the *TP53* locus: 7 were CN-LOH and 12 were deletions (Figure 1A). DNA was available for 15/19 of these patients. We found homozygous *TP53* mutations in 5/6 patients (83%) with somatic CN-LOH and hemizygous *TP53* mutation in 7/9 patients (78%) with del(17p) (Figure 1D). Of 12 *TP53* mutations found, 11 (92%) were missense and located in the DNA-binding core domain (residues 102–292) of *TP53* (4 in exon 5: C141Y; V172F; C176Y; H179Q; 2 in exon 6: H193N, H193R; 1 in exon 7: R249G and 4 in exon 8: V272L, V272M, 2R273H twice; Table 13). One homozygous frameshift/nonsense mutation was found in exon 9. Overall, *TP53* mutations were found in 12 of 15 cases (80%) with LOH in the *TP53* locus. To compare the rate of *TP53* mutations in MDS/AML patients with and without chromosome 17 aberrations by either MC or SNP-A, we screened an additional 40

patients with MDS or AML. Five had a normal karyotype, 14 had a complex karyotype (9/14 with aberrations of chromosomes 5, 7, or both; and 5/14 without aberrations of 5 or 7), 7 had sole -7/7q-, 4 had sole 5q- and 10 had -5/5q- or -7/7q- with other abnormalities. We found only two heterozygous mutations (M273I, R248Q) in these patients; in both cases the patients had a complex karyotype with -5/5q- and -7/7q- without LOH17p. Of interest, one of these patients transformed from aplastic anemia (AA) to AML. For this patient we were able to partially reconstruct the time of mutation occurrence. Five months after diagnosis with AA, SNP-A detected a minor clone with monosomy 7. At this time there was no sign of *TP53* mutation. Two years after AA diagnosis the patient developed AML. At this time, analysis of bone marrow by MC and SNP-A showed a complex karyotype and a heterozygous mutation of *TP53* was found.

In our cohort of 379 patients 11.6% of cases had a complex karyotype (in our study complex karyotype was defined as three or more chromosomal aberrations) and, of these, 45% also had LOH17p. Only one patient without a complex karyotype had LOH17p. In cases with a complex karyotype and LOH17p, -5/5q- and/or -7/7q-, *TP53* mutations were frequent (75%). *TP53* mutations were much less frequent (2/40; 5%) in patients without aberrations of chromosome 17 by either MC or SNP-A (Figure 1D). These results are concordant with previous report showing complete or partial loss of 17p in 4.3% of 1138 MDS/AML patients. In 69% of those patients a *TP53* mutation was found as compared to a frequency of *TP53* mutation of 3.1% in patients without abnormalities of chromosome 17[2].

Our findings emphasize the validity of Knudson's classic two-hit model of tumorigenesis in which tumor suppressor genes such as *TP53* must undergo inactivation of both alleles to permit tumor formation[4]. In this context, we propose two mechanisms by which both *TP53* alleles may be inactivated. In the case of deletion 17p, one allele becomes inactivated by mutation and the wild-type allele is lost through deletion. In patients with CN-LOH17p, one more step can be considered: reduplication of the mutated *TP53* allele as a result of an attempt to correct for the lost chromosomal material. Indeed, this mechanism has been proposed to explain homozygous *TP53* mutations in patients with apparently normal chromosomes 17 and two FISH signals for *TP53*[5]. Our results suggest that intragenic *TP53* mutation preceded LOH17p, based on the fact that we found two patients with heterozygous *TP53* mutations without the presence of LOH17p by MC or SNP-A. It is likely that such patients would show disease progression associated with subsequent 17p deletion or somatic CN-LOH17p, either of which would lead to loss of the protective wild-type allele.

A high frequency of *TP53* mutations in at least a partially heterozygous constellation has been described in AML with a complex karyotype[2;6–7]. However, since neither SNP-A nor microsatellite analyses were performed in these studies, we suggest an alternative explanation: that identical findings could occur if DNA were isolated from a mixture of normal and abnormal cells, where a clone of cells with CN-LOH17p is the source of the homozygous mutated allele and admixed normal cells are the source of the wild-type allele. We and others[2;6–7] have shown that *TP53* mutations occur frequently in MDS/AML patients with complex karyotype, especially with del17p, del5/5q and del7/7q and are very rare in patients with other cytogenetic abnormalities. Our results point towards the presence of homozygous mutations in the context of CN-LOH17p while previous studies

demonstrated the presence of hemizygous mutations. Our study explains why in the former studies patients with del5/5q- and del7/7q- showed *TP53* mutations without obvious del17p: they may have had CN-LOH17p. We have proceeded to sequence patients without del17 (with and without del5/5q and del7/7q) and found 2 mutations in 40 (5%) patients; in a previous work in which such patients were sequenced, only 8/256 (3.1%) mutations were found, likely associated with CN-LOH17p for which they were not screened2. A high incidence of *TP53* mutation in MDS/AML patients with complex cytogenetics with deletions of chromosomes 5, 7 and 17 suggests a cooperative effect between these lesions. This non-random pattern of DNA losses in MDS/AML patients with a complex karyotype is of interest, and suggests the presence of a multi-step pathway which is responsible for the sequential accumulation of certain chromosomal aberrations that results in poor clinical outcome8.

In sum, our study demonstrates that CN-LOH17p is a frequent type of cytogenetic defect occurring in patients with advanced MDS and sAML with a complex karyotype and is strongly associated with homozygous *TP53* mutations. As described in previous reports, hemizygous deletions affecting this locus have been associated with mutation in the remaining *TP53* allele. Previously, detection of *TP53* mutations in patients without a visible cytogenetic deletion in this region prompted the assumption that these mutations were heterozygous2;6–7. However, our study indicates that in MDS/sAML, *TP53* mutations can occur in either a hemizygous or homozygous constellation and heterozygous mutations are actually very rare as previously reported in large cohort of patients2. Clinically, prognosis of patients with CN-LOH17p is as poor as that of patients with del17p. LOH17p is strongly associated with a complex karyotype in combination with whole or partial losses of chromosomes 5 and 7, and hemi- or homozygous *TP53* mutations. Complex karyotypes with 5q and 7q abnormalities without del(17p) often harbor CN-LOH17p, a lesion which is cryptic unless analyzed by SNP-A.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

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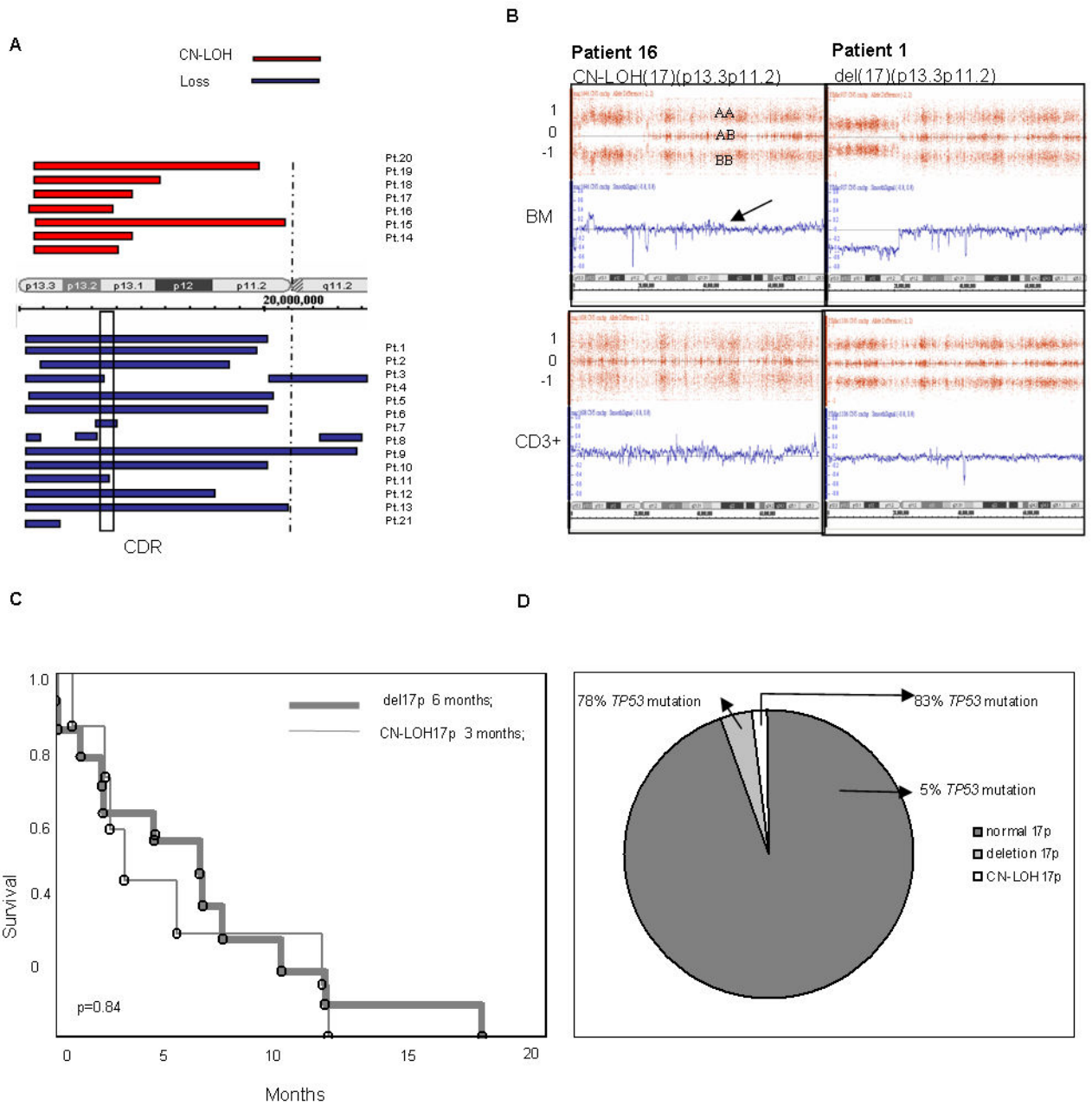
Figure 1.

(A) Summary of LOH17p detected by SNP-A. Lesions of chromosome 17p detected by SNP-A for individual patients are shown (N=21). Blue bars indicate deleted regions. Red bars designate areas of acquired somatic copy neutral loss of heterozygosity (CN-LOH). The commonly deleted region (CDR) is marked by black lines. (B) 6.0 SNP-A "karyograms" of whole bone marrow and CD3+ lymphocytes for two representative patients show the somatic origin of acquired CN-LOH and deletion of chromosome 17p. Red dots (The Allele Difference graph) illustrate the genotypes for each individual SNP. Dots with a value of 1 represent SNPs with an "AA" genotype; those with a value of –1 indicate SNPs with "BB"

genotype and dots at 0 represent heterozygous "AB" SNPs. Complete loss of all SNPs with "AB" genotype indicates regions of LOH. The blue line (black arrow) represents log2 ratio between patient sample and reference signal intensity. In the bone marrow of patient 16, SNP-A detected copy-neutral LOH (17)(p13.3p11.2) designated by loss of "AB" SNPs and no changes in log2 ratio. For patient 1, bone marrow SNP-A detected a deletion (17) (p13.3p11.2) indicated by loss of "AB" SNPs, reduction in allele difference and log2 ratio. In sorted CD3+ lymphocytes of patient 16 and 1, a normal chromosome 17 is seen. (C) Kaplan-Meier analysis of survival of patients with CN-LOH17p and del(17p). (D) Distribution of *TP53* mutation in MDS/AML patients with and without LOH17p

Table 1

Characteristics of *TP53* mutations found in patients with LOH17p identified by SNP-A

Patient No.	SNP-A	Exon	Codon			Mutation	Structural Motif*	Frequency in hematologic malignancies*
			No	Wt	Mutant			
1	del17p	8	273	CGT	CAT	R273H	S10	3%
4	del17p	6	193	CAT	AAT	H193N	L2	NA
6	del17p	7	249	AGG	GGG	R249G	L3	NA
7	del17p	5	179	CAT	CAG	H179Q	H1	NA
9	del17p	6	193	CAT	CGT	H193R	L2	0.6%
11	del17p	5	176	TGC	TAC	C176Y	L2	0.8%
13	del17p	8	273	CGT	CAT	R273H	S10	3%
14	CN-LOH17p	5	172	GTT	TTT	V172F	L2	NA
15	CN-LOH17p	8	272	GTG	CTG	V272L	S10	NA
16	CN-LOH17p	8	272	GTG	ATG	V272M	S10	0.9%
19	CN-LOH17p	5	141	TGC	TAC	C141Y	S3	0.5%
20	CN-LOH17p	9	309			P309fsx28		NA

Abbreviations: NA - not available

*based on: IARC *TP53* database (http://www-p53.iarc.fr/)3.