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ORIGINAL ARTICLE

Mutations of the *TP53* gene in acute myeloid leukemia are strongly associated with a complex aberrant karyotype

C Haferlach, F Dicker, H Herholz, S Schnittger, W Kern and T Haferlach

MLL Munich Leukemia Laboratory, Max-Lebsche-Platz, Munich, Germany

In acute myeloid leukemia (AML) with complex aberrant karyotype, a loss of one *TP53* allele is frequently observed. We analyzed the incidence of *TP53* mutations and deletions in 107 AML with complex aberrant karyotype. In 50 of 57 cases showing a loss of one *TP53* allele, a *TP53* mutation was detected in the remaining allele. In addition, in 33 of 50 cases with two *TP53* copies, a *TP53* mutation was found. Therefore, the frequency of *TP53* mutations in AML with complex aberrant karyotype was 78%. In a second step, we analyzed *TP53* mutations in a cohort of AML comprising different cytogenetic subgroups. *TP53* mutations were detected in 33 of 235 cases (14%). Coincidences with other molecular mutations were rare. We confirmed a high incidence of *TP53* mutations in AML with a complex aberrant karyotype (29/42, 69%) and demonstrated that *TP53* mutations are very rare in AML without a complex aberrant karyotype (4/193, 2.1%).
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Introduction

The *TP53* gene is the most frequently mutated gene in human tumors identified so far.[1] In contrast, the reported *TP53* mutation rate in acute myeloid leukemia (AML) is low.[2–5] However, in AML with complex aberrant karyotype, a loss of the short arm of chromosome 17 is frequently observed, where the *TP53* gene is located.[6] In a previous series, we showed a loss of one copy of the *TP53* gene in 66 of 125 cases with AML and a complex aberrant karyotype (52.8%) by fluorescence *in situ* hybridization (FISH).[7] According to the classical tumor suppressor gene model, it can be assumed that mutations in the remaining allele are frequent. Therefore, the aim of this study was to determine the frequency of *TP53* deletions and mutations in AML with a complex aberrant karyotype. In addition, we evaluated a cohort of AML representing all frequent cytogenetic subgroups for their respective frequency of *TP53* mutations and analyzed the relation to other molecular genetic aberrations.

Patients and methods

Patients

After the informed written consent was obtained, bone marrow or peripheral blood samples, which were referred to our laboratory were analyzed with chromosome banding analysis, interphase FISH and for molecular mutations. Two different cohorts were analyzed: (1) 107 cases with AML and a complex aberrant karyotype defined as showing three or more clonal abnormalities but no balanced rearrangement leading to a leukemia-specific fusion gene; (2) 235 AML cases (214 *de novo* AML, 13 therapy-related AML (t-AML) and eight AML evolving from a myelodysplastic syndrome (s-AML)) comprising cases of all frequent cytogenetic categories (Table 1).

Metaphase cytogenetics

Cultivation and chromosome preparation was carried out as described elsewhere. In each case with a complex aberrant karyotype, results of G-banding analysis were confirmed by 24-color FISH. Chromosomes were classified according to the International System for Human Cytogenetic Nomenclature (ISCN, 1995).[8]

Fluorescence in situ *hybridization*

Interphase FISH with a probe for the *TP53* gene (ABBOTT, Wiesbaden, Germany) was performed in all cases with a complex aberrant karyotype as described previously.[7]

TP53 *mutation analysis*

TP53 mutation analysis was carried out using the Affymetrix p53 GeneChip assay, denaturating high performance liquid chromatography and direct sequencing. Exons 3–4, 5–6, 7 and 8–9 of the *TP53* gene were amplified by PCR from genomic DNA (200 ng) and subsequently analyzed by denaturating high performance liquid chromatography on the WAVE-system (Transgenomic Inc., Omaha, Nebraska, USA). Aberrant amplicons were verified by direct sequencing in both orientations using Big Dye Chemistry (Applied Biosystems, Weiterstadt, Germany) and analyzed on a 3130 Genetic Analyzer (Applied Biosystems). In all 24 cases without a *TP53* mutation observed by the Affymetrix p53 GeneChip assay or denaturating high performance liquid chromatography in the first cohort of 107 cases with a complex aberrant karyotype, direct sequencing of exon 3–9 and intron 2–9 was carried out.

Mutations analysis within FLT3, NPM1 *and* MLL

Fms-related tyrosine kinase 3 (*FLT3*) length mutation, mixed-lineage leukemia (*MLL*) partial tandem duplication and nucleophosmin (*NPM1*) mutation analysis was performed as published elsewhere. All cases in cohort 2 were analyzed for all three mutations.

Results and discussion

The first cohort analyzed comprised 107 AML cases with a complex aberrant karyotype. *TP53* deletions were detected by

Correspondence: Dr C Haferlach, MLL Munich Leukemia Laboratory, Max-Lebsche-Platz 31, 81377 Munich, Germany.
E-mail: claudia.haferlach@mll-online.com
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Table 1 Type of *TP53* mutation detected in 107 cases with AML and complex aberrant karyotype (cohort 1)

	Total	*Cases with the loss of one* TP53 *copy*	*Cases with two* TP53 *copies*
Missense mutation	73	46	27
Nonsense mutation	6	3	3
Deletion	2	0	2
Insertion	2	2	0
Splice site mutation	7	2	5
	90	53	37

Abbreviation: AML, acute myeloid leukemia.

interphase FISH in 57 cases, while 50 cases showed two copies of *TP53* in FISH analysis. *TP53* mutations were detected in 50 of 57 cases showing a loss of one *TP53* allele (88%). In addition, 33 cases (66%) without a *TP53* loss showed a *TP53* mutation. Therefore, the overall frequency of *TP53* mutations was 78% in AML with complex aberrant karyotype. In total, 90 mutations were detected in 83 cases. Two mutations within the *TP53* gene were found in four cases with two *TP53* copies and in three cases with loss of one *TP53* allele. Overall, only 17 of 107 cases (16%) showed neither loss nor mutation of *TP53*. The type of *TP53* mutation detected in 107 cases with AML and complex aberrant karyotype is depicted in Table 1.

In cohort 2 comprising 235 AML cases, *TP53* mutations were detected in 33 of the 235 cases (29 *de novo* AML, two t-AML, and two s-AML) (14%). *FLT3*–LM was observed in 33 (14%), *MLL*–PTD in eight (3.4%), *NPM1* mutations in 43 cases (18.3%), respectively. In one case each, a *TP53* mutation occurred in combination with an *FLT3*–LM or *MLL*–PTD, respectively. A *TP53* mutation was not detected together with an *NPM1* mutation. Suzuki *et al.*[9] reported on two cases carrying both a *TP53* and an *NPM1* mutation out of 60 cases with *NPM1* mutation (3.3%).

A complex aberrant karyotype was present in 29 of 33 cases (87.9%) with a *TP53* mutation. The remaining four cases with *TP53* mutation showed a normal karyotype, a trisomy 8, trisomy 13 and t(8;21), each aberration occurring as the sole abnormality, respectively. Therefore, we confirmed a high incidence of *TP53* mutations in AML with complex aberrant karyotype (29/42, 69%). On the other hand, *TP53* mutations are very rare in AML without a complex aberrant karyotype (4/193, 2.1%).

The frequency and association between *TP53* mutations and karyotype, *FLT3* length mutations, *NPM1* mutations and partial tandem duplications within the *MLL* gene in cohort 2 ($n=235$) are shown in Table 2. A close association was observed between *TP53* mutations and a complex aberrant karyotype, while *TP53* mutations were nonrecurrent in all other cytogenetic subgroups. In addition, *TP53* mutations occurred only rarely in combination with *FLT3* length mutations and partial tandem duplications of the *MLL* gene and were not observed together with *NPM1* mutations, which are strongly associated with AML and normal karyotype. Therefore, a close correlation of cytogenetic abnormalities and molecular mutations is confirmed.

Furthermore, we divided AML with complex aberrant karyotype into two subgroups: (1) AML with 'typical' complex aberrant karyotype showing a deletion of at least one of the following regions: 5q31, 7q31, 17p13 as previously proposed and (2) AML with 'untypical' complex aberrant karyotype comprising all others.[7] The frequency of *TP53* mutations within the typical complex aberrant karyotype group was 76.0% (20/26), while in the untypical group, it was 56.2% (9/16; $P=0.16$).

Table 2 Association between *TP53* mutations and karyotype, *FLT3* length mutations, *NPM1* mutations and partial tandem duplications within the *MLL* gene in 235 AML cases (cohort 2)

Cytogenetic subgroup	n	TP53-*Mut*+	FLT3-*LM*+	*NPM*+	MLL-*PTD*+
Normal karyotype	75	1	17	31	5
t(8;21)	15	1	0	0	0
t(15;17)	10	0	2	0	0
inv(16)/t(16;16)	11	0	1	0	0
t(11q23)/*MLL*	13	0	0	0	0
complex	42	29	2	1	1
+13 sole	10	1	3	0	1
+8 sole	14	1	4	4	1
−7 sole	4	0	0	0	0
t(6;9)	3	0	1	0	0
inv(3)/t(3;3)	2	0	0	0	0
del(5q) sole	2	0	0	0	0
del(20q) sole	1	0	0	0	0
−Y sole	1	0	0	1	0
Other abnormalities	32	0	3	6	0

In therapy-related myelodysplastic syndrome (MDS) and AML, an association between 5q deletions, a complex aberrant karyotype and *TP53* mutations were reported.[10,11] In our series in both cases with 5q deletion as the sole abnormality, no *TP53* mutation was observed. In AML with a complex aberrant karyoytpe showing a 5q deletion, the *TP53* mutations rate was 76% (19/25) as compared to 58.8% (10/17) in cases with complex aberrant karyotype without 5q deletion ($P=0.237$).

Conclusions

In conclusion, in AML, *TP53* mutations are highly associated with AML and complex aberrant karyotype and occur very infrequently in all other cytogenetic subgroups ($P<0.001$). They occur frequently, in particular, in the subgroup showing a typical pattern of chromosomal deletions (5q, 7q and 17p). *TP53* mutations might explain in part the poor prognosis due to chemoresistance of AML with complex aberrant karyotype, which has been observed consistently in clinical trials.[12–16] In addition to cytogenetics, a rapid diagnostic screening for *TP53* mutations could be a valuable tool to identify a subgroup of AML with poor prognosis. This would allow the early assignment of patients to alternative treatment strategies using options targeting the *TP53* pathway.

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