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Significance of chromosomal alterations and mutations of the N-*RAS* and *TP53* genes in relation to leukemogenesis of acute myeloid leukemia

Shinichi Misawa [a,b,*], Shigeo Horiike [b], Hiroto Kaneko [b], Yuri Sasai [b], Yutaka Ueda [b], Makoto Nakao [b], Shouhei Yokota [b], Masafumi Taniwaki [b], Hiroshi Fujii [c], Hitoshi Nakagawa [c], Shouichirou Tsuda [d], Kei Kashima [b]

[a] *Division of Hematology, Kyoto Prefectural Yosanoumi Hospital, 481 Otokoyama, Iwatakicho, Yosagun, Kyoto-fu 629-2261, Japan*
[b] *Third Department of Medicine, Kyoto Prefectural University of Medicine, Kawaramachi-Hirokoji, Kamigyo-ku, Kyoto 602-0841, Japan*
[c] *Third Department of Medicine, Kyoto First Red Cross Hospital, Hon-cho, Higashiyama-ku, Kyoto 605-0981, Japan*
[d] *Division of Hematology/Oncology, Aiseikai-Yamashina Hospital, Takehana, Yamashina-ku, Kyoto 607-8086, Japan*

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Abstract

We examined chromosomes and mutations of the N-*RAS* and *TP53* genes in 73 patients with acute myeloid leukemia (AML). Twenty-six patients showed a reciprocal chromosomal translocation or an inversion, and 34 patients showed only unbalanced aberrations. Balanced aberrations were predominantly detected in the AML patients who did not have myelodysplasia, preceding myelodysplastic syndrome, and a history of chemotherapy or radiation therapy. In contrast, unbalanced aberrations were more frequently seen in the patients with AML with trilineage myelodysplasia, AML transformed from MDS, and therapy-related AML. Twenty-two mutations of the N-*RAS* and *TP53* genes were detected, and these mutations were frequently associated with unbalanced chromosomal aberrations. Furthermore, the spectrum of mutations was suggestive of an exposure to alkylating chemicals. © 1998 Elsevier Science Ltd. All rights reserved.

Keywords: AML; Secondary leukemia; Chromosomes; N-*RAS* mutation; *TP53* mutation; Multistep carcinogenesis

1. Introduction

The multistep model of carcinogenesis is well defined in human cancers, such as colon cancer [1], therapy-related acute myeloid leukemia (t-AML) and myelodysplasia [2]. Acute myeloid leukemia (AML) may arise de novo from unknown causes and may present without the preleukemic phase, i.e. myelodysplastic syndrome (MDS). Cytogenetically, balanced translocations predominate over other aberrations [3]. A recombination of genes through these translocations has been demonstrated in most of these classes of chromosomal alterations [4].

Therapy-related AML (t-AML) now accounts for up to 20% of all AML cases [5]. The classical form of t-AML is characterized by known prior exposure to alkylating agent chemotherapy or irradiation [5,6]. Frequent chromosomal loss or deletion, particularly involving chromosomes 5 and 7, suggests that loss of heterozygosity for specific alleles could be an important step in leukemogenesis [7,8]. The other form of t-AML, arising after chemotherapy with epipodophyllotoxins, presents abnormalities involving chromosome 11 at the 11q23 band [5,6]. Therapy-related leukemia has also been shown to related to other drugs targeting topoisomerase II (topo II), doxorubicin or mitoxantron, and is

Abbreviations: AML, acute myeloid leukemia; AML/TLD, de novo AML with trilineage myelodysplasia; MDS, myelodysplastic syndrome; p-AML, de novo AML in which there was no previous history of chemotherapy or radiation therapy, no preceding MDS phase, and no myelodysplasia. AML/TLD and AML transformed from MDS are excluded from this category, as is t-AML; PCR, polymerase chain reaction; t-AML, therapy-related AML; topo II, topoisomerase II.

* Corresponding author. Kyoto Prefectural Yosanoumi Hospital, 481 Otokoyama, Iwatakicho, Yosagun, Kyoto-fu, 629-2261 Japan. Tel./fax: +81 772 463371.

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associated with reciprocal translocations of t(8;21), t(15;17), or inv(16) [5,6].

There is a proportion of de novo AML patients (approximately 15%) in whom neither a history of chemo- or radiation therapy nor a preleukemic phase was present, but in whom characteristic features of dysplastic hematopoiesis were observed in the bone marrow. This type of leukemia is designated de novo AML with trilineage myelodysplasia (AML/TLD) [9], and some of these patients may have a history of regular exposure to various chemicals. These findings suggest the possibility of secondary leukemia due to an exposure to known or unknown mutagens or carcinogens, or of an advanced form of MDS [10]. Although the progression to overt AML from the preceding MDS phase represents the outgrowth of a newly evolved leukemia clone, limited evidence of a multistep process of malignant transformation has been obtained [2,11–13].

The increasing incidence with age of MDS and AML with unbalanced chromosomal aberrations could be related to a life-long exposure of the general population to chemical carcinogens [14] and may follow the multistage model of leukemogenesis. In contrast, the less age-dependent development of AML with balanced chromosomal aberrations may be due to an alternative mechanism, for instance, spontaneous illegitimate recombination during the normal activity of topo II [5,6]. We examined the chromosomes and mutations of the N-*RAS* and *TP53* genes in 73 patients with the above-described categories of AML to elucidate the roles of these genetic alterations in human leukemogenesis.

2. Materials and methods

2.1. Patients

We studied 73 patients with AML whose chromosomes were fully karyotyped and whose cell pellet or DNA had been preserved to examine the status of the N-*RAS* and *TP53* genes. The diagnoses of AML and MDS were made according to the FAB classification [10,15] and that of AML/TLD was made according to the proposal of Brito-Babapulle et al. [9]. Five patients were diagnosed as having t-AML, which was defined by a previous history of chemotherapy or radiation therapy. Among the remaining 68 patients with de novo AML, 20 had AML transformed from MDS by a presence of preceding MDS phase, and 17 had AML/TLD. A consecutive series of patients with t-AML, AML transformed from MDS, and AML/TLD were subjected to study. We also examined 31 patients with de novo AML who had no previous history of chemotherapy or radiation therapy, no preceding MDS phase, and no myelodysplasia as a control group; they are referred to as the primary overt AML or p-AML patients in this report. In this control group of patients with AML, patients with AML/TLD and AML transformed from MDS were excluded in addition to those with t-AML. Patients with p-AML were selected randomly, but a few more cases with balanced chromosomal translocations were included for statistical analysis since balanced translocations were relatively rare cytogenetic events in the other groups of patients.

Table 1
Type of acute myeloid leukemia and chromosomal findings

Type of AML	Karyotype			Total
	Normal	Abnormal[a]		
		Balanced	Unbalanced	
AML, primary overt	7 (23%)	19 (61%)	5 (16%)	31
AML/TLD	2 (12%)	3 (18%)	12 (71%)	17
AML, transformed from MDS	4 (20%)	3 (15%)	13 (65%)	20
AML, therapy-related	0 (0%)	1 (20%)	4 (80%)	5
Total	13 (18%)	26 (36%)	34 (47%)	73

[a] Balanced includes cases exhibiting any balanced aberrations regardless of co-existing unbalanced aberrations, and unbalanced includes cases with only unbalanced aberrations.

2.2. Cytogenetic analysis

Bone marrow samples were obtained after obtaining the informed consent of the patients. The chromosomes

Table 2
Summary of chromosomal abnormalities detected in 60 patients with an abnormal karyotype

Balanced, 26 Cases[a] (simple 23, complex 3)		Unbalanced, 34 Cases[b] (simple 16, complex 18)	
t(8;21)(q22;q22)	8	−5/5q−	13
t(15;17)(q22;q21)	8	−7/7q−	12
t(1;7)(q32;q32)	1	−12/12p−	9
t(1;10)(q22;p13)	1	−17/17p−	11
t(3;5)(q21;q31)	1	+1q	2
t(3;12)(q25;p11)	1	+8	4
t(5;12)(q33;p13)	1	+21	5
t(7;11)(p15;p15)	1	—	—
t(9;11)(p22;q23)	1	—-	—
t(9;17)(q22;p13)	1	—	—
t(9;22)(q34;q11)	1	—	—
inv(16)(p13q22)	1	Several abnormalities are overlapped	

[a] Cases in which any of the reciprocal translocations or inversion were detected.
[b] Cases with only unbalanced chromosomal aberrations are listed. Simple, one to two aberrations; complex, three or more aberrations in the mainline.

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Table 3
AML patients with an N-*RAS* mutation

Case	Age/sex	FAB[b]	Mutation (codon)[c]	Karyotype
809	50/F	M2*	GTT (13)	48,XX,+21,+add(21)(p13)[5]
601	58/M	M4/TLD*	GCT (12), GTT (13)	46,XY,del(12)(p12p13)[3]/N[5]
606	49/M	M4/TLD*	CTA (61)	46,XY,t(7;11)(p15;p15)[4]
607	75/F	M4/TLD*	TGT (12)	47,XX,+mar[3]/N[5]
610[a]	71/M	M4/TLD*	TGT (12)	45,XY,t(9;17)(q22;p13),−17,dmin[6]/N[6]
612[a]	82/F	M4/TLD*	GTT (12)	46,XX,del(5)(q13q31)[4]/idem,-7,add (16)(q22),add(21)(q22)[3]/idem,add(17)(p11)[11]/N[4]
338	78/F	RAEB→M2*	GAT (13)	46,XX,del(12)(p11p13)[12]/N[8]
304	60/M	RAEB** →M2*	GAT (13)	46,XY,der(20)t(8;20)(q22;p13)[15]
344	63/M	RAEB→M2*	GTT (12)	52,XY+8,inv(9)(p11q13)c,+11,+13,+18,+19,+21[4]/N[8]
112	48/M	RA→M4*	GAT (12)	46,XY,der(3)t(3;8)(q29;q13)[1]/idem,del(11)(q21q25)[23]

The karyotype and N-*RAS* mutation of cases 338, 304, 344, and 112 were reported as cases 32, 31, 33, and 28, respectively, by Horiike and co-workers [12].
[a] *TP53* mutation was also detected.
[b] A single asterisk (*) designates samples in which an N-*RAS* mutation was detected; a double asterisk (**) indicates that N-*RAS* was not examined.
[c] Wild-type sequences of codons 12 and 13 are GGT and 61 CAA.

were examined by our standard method using G-banding [3] and designated according to the International Cytogenetic Nomenclature (1995) [16]. We counted a balanced translocation as one aberration, a gain or loss of a whole chromosome or a deletion as one, and an unbalanced translocation as two, and the total number of chromosomal abnormalities observed in the karyotype of the mainline [16] was categorized as simple (one or two aberrations) or complex (three or more).

2.3. Mutations of the N-RAS and TP53 genes

The samples were screened for the presence of mutations of N-*RAS* at codons 12 and 13 by polymerase chain reaction (PCR) used to insert a *Bst*NI or *Pfl*MI restriction enzyme site. In brief, after amplifying a 328-basepair (bp) genomic fragment including exon 1 of the N-*RAS* gene, a second round of PCR was performed using nested primers in which a novel restriction site was introduced. After digestion with the corresponding endonucleases (*Bst*NI for codon 12 and *Pfl*MI for codon 13), the second-round products were resolved by electrophoresis on a 3% agarose gel. To detect mutations of the N-*RAS* gene at codon 61 and those of the *TP53* gene in exons 4–8, we performed a single-strand conformation polymorphism analysis by means of PCR. After amplifying 100 ng of genomic DNA using corresponding sets of primers and [α-^{32}P]dCTP, the products were diluted, denatured and resolved by electrophoresis on a 6% polyacrylamide gel, followed by exposure to Kodak X-Omat films at −80°C. The presumptive mutations were confirmed by direct sequencing with the dideoxy-mediated chain-termination method using ^{32}P-labeled sequence primers and a T7 Sequencing Kit (Pharmacia LKB, Upssala, Sweden). In cases which showed a deletion of sequences of *TP53*, we cloned the PCR products into the pCR 1000 vector using a TA cloning Kit (Invitrogen Co., San Diego, CA) and sequenced them.

The sensitivity of these procedures was 5×10^{-2}, which means that approximately 5% of the cells with the mutation could be detected. The details of the assay systems are described elsewhere [12,13,17].

2.4. Statistical analysis

We compared the incidence of genetic alterations between groups of patients (one represented bone marrow dysplasia and the other did not), and between those who had balanced chromosomal aberrations and those with only unbalanced ones. The statistical analysis was performed using Fisher's exact probability test with Yates' continuity correction. Probability values less than 5% were considered significant.

3. Results

3.1. Cytogenetic results

Balanced chromosomal alterations were detected in 26 (36%) of the patients examined; 15–20% of the patients with AML/TLD, AML transformed from MDS, or t-AML, and 61% of the patients with p-AML

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Table 4
AML patients with a *TP53* mutation

Case	Age/sex	FAB[b]	Mutation (codon)	Karyotype
603	69/F	M2/TLD (R*)	GAG-TAG (224)	45,XX,+3,-5,add(7)(q32),-17,-18,+mar[11]/idem,+8,+9[9]/N[3]
604	80/M	MO/TLD*	ATG-GTG (246)	45,XY,−5,der(5)t(5;12)(p13;q25),−7,del(12)(q22q24),−17,−20,+2mar,+r[8]
605	59/F	M2/TLD (R*)	TAT-TGT (220)	45,X,-X,t(3;5)(q21;q31)[2]/N[3]
610[a]	71/M	M4/TLD*	g-a (intron 4)	45,XY,t(9;17)(q22;p13),−17,dmin[6]/N[6]
612[a]	82/F	M4/TLD*	TGC-TAC (176)	46,XX,del(5)(q13q31)[4]/idem,-7,add(16)(q22),add(21)(q22)[3]/idem,add(17)(p11)[11]/N[4]
129	31/F	RA*→M6*	TGT-TTT (277)	45,XX,del(5)(q22q35),−13[6]/N[3]
340	66/M	RAEB*→M2*	TGC-TAC (242)	44,XY,add(5)(q11),-7,der(12)t (1;12)(p32;p13),-17,add(21)(p13)[3]/N[5]
525	76/M	RAEB-T*→M2*	CAT-CTT (193)	45,XY,add (5) (q11),−18,add (19) (p13)[10]
530	65/M	RAEB-T*→M6*	CCCTCC-C (89,90)	44,XY,t (1;10) (q21;p13), −5,ins (12) (q11;?),add (17) (p13),−18,dmin[3]/idem, +21[3]/N[1]
140	59/M	RA*→M4*	CTT-CT (194)	45,XY,del(1)(q12),del(5)(q13q31),−7,add (11) (q13),−12,−15,add(17)(p11),+21[7]/N[1]
701	38/M	t-M5 (R*)	GTG-GTA (197)	46,XY,add(7)(q22),t(9;11)(p22;q23)[3]/N[5]
337	76/M	t-M4*	CAA-TAA (136)	44,XY,del(5)(q22q35),dic(7;12)(q11;p11),i(11q),dic(11;16)(p11;q22),−13, −15,add(17)(p13)[9]/N[2]

The karyotype and *TP53* mutation of cases 129, 340, 525, and 337 were reported as cases 5, 6, 7, and 4, respectively, by Kaneko and co-workers [13].
[a] N-RAS mutation was also detected.
[b] An asterisk (*) designates samples in which a *TP53* mutation was detected, and an (R) *TP53* mutation was detected at relapse but not at presentation.

(Table 1). The other 34 patients displayed unbalanced aberrations without any balanced aberrations. There were 11 reciprocal translocations and one inversion observed in 26 patients (Table 2). In these patients, one reciprocal translocation or an inversion was detected as a sole abnormality in 17 patients, associated with −Y in four, +8 in two, and with three or more aberrations in three. Among the 34 patients with solely unbalanced aberrations, loss of all or part of chromosome 5 was observed in 13 patients, chromosome 7 in 12, chromosome 12 in nine, and chromosome 17 in 11. In addition, these losses frequently overlapped; thus, 12 patients had a single aberration, four patients two aberrations, and 18 patients three or more aberrations.

3.2. N-*RAS* mutation

An N-*RAS* mutation was detected in 10 patients (Table 3). One patient had two N-*RAS* mutations (case 601) [17], and two others had a *TP53* mutation as well (cases 610 and 612). Among the four patients with AML transformed from MDS, three patients were also examined in their MDS phase with respect to the presence of the N-*RAS* mutation, but the result was negative [12]. The chromosomal alterations seen in the 10 patients were simple in five patients and complex in the remaining five.

3.3. *TP53* mutations

A *TP53* mutation was detected in 12 patients; 10 patients showed a point mutation and two a 1- or 5-bp deletion (Table 4). Two of the patients had an N-*RAS* mutation as well. Among these 12, one patient each had a t(3;5), t(9;11), or t(9;17) reciprocal translocation with one or two additional chromosomal aberrations, one had a 5q- and a -13, and the remaining eight showed a complex aberration including at least one of the deletions listed in Table 2. All five of the patients with MDS who displayed a *TP53* mutation also showed the same mutation at the time of diagnosis of MDS (2–7 months before the manifestation of AML) [13]. In contrast, two patients with AML/TLD and one with t-AML harbored a *TP53* mutation at their relapse, and the initial samples obtained at the presentation of AML were negative for the mutation. Regarding the loss of the short arm of chromosome 17, eight patients showed a 17p abnormality.

3.4. Type of AML, chromosomal findings, and genetic alterations

At least one of the genetic alterations of chromosomal abnormalities or mutations of the N-*RAS* or *TP53* gene was detected in 60 (82%) of the 73 patients examined, a similar incidence among the four categories of AML (Table 5). A mutation of the N-*RAS* gene was

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Table 5
Summary of type of AML and genetic alterations

Type of AML	No. of cases	Abnormal karyotype	N-*RAS* mutation	*TP53* mutation
AML, primary overt	31	24 (77%)	1 (3%)	0 (0%)
AML/TLD	17[a]	15 (88%)	5 (29%)	5 (29%)
AML, transformed from MDS	20	16 (80%)	4 (20%)	5 (25%)
AML, therapy-related	5	5 (100%)	0 (0%)	2 (40%)
Total	73	60 (82%)	10 (14%)	12 (16%)

[a] One case showed two N-*RAS* mutations and two others both N-*RAS* and *TP53* mutations.

detected in five (29%) and four (20%) patients with AML/TLD and AML transformed from MDS, respectively, but in one (3%) of the 31 control patients with p-AML and in none of the five with t-AML. *TP53* mutations were found in two (40%) patients with t-AML, in 29% and 25% of the patients with AML/TLD and with AML transformed from MDS, respectively, but in no patients with p-AML. Consequently, both of these gene mutations were a rare genetic event in p-AML but were consistent in the other groups of patients, except that the N-*RAS* gene mutation was rare in t-AML.

In summary, balanced chromosomal alterations were more frequently observed in p-AML, and genetic alterations of the N-*RAS* or *TP53* genes were associated with an abnormal karyotype, especially with unbalanced chromosomal alterations (Table 6). These mutations were not detected in any patients with a normal karyotype. When N-*RAS* and *TP53* mutations were compared with respect to the total number of chromosomal abnormalities, *TP53* mutation was found to be associated with a complex karyotype and a karyotype including one of the deletions predominantly observed (Table 7). Single-variate analysis showed a correlation between *TP53* mutation and loss of all or part of chromosomes 5, 7, and 17, among the four most frequent losses in our study. Multi-variate analysis showed that loss of all or part of chromosome 5 contributed most to the *TP53* mutation by discriminant analysis. In contrast to *TP53* mutation, N-*RAS* mutation did not correlate with any of the above-mentioned chromosomal categories.

4. Discussion

Among the 73 patients examined, 26 showed one of the balanced aberrations. The karyotypes including a balanced aberration were rather simple and were observed more frequently in the patients with primary overt AML. These alterations may have contributed to leukemogenesis by the formation of abnormal chimeric genes; nine of the 12 balanced aberrations observed in our patients have been shown to reconstruct a recombination of genes [4,18–20]. In contrast, the karyotypes including only unbalanced aberrations (observed in 34 patients) were rather complex; 18 patients had three or more aberrations. Unbalanced aberrations were frequently detected in patients with AML/TLD, AML transformed from MDS, and t-AML. Deletions of all or parts of chromosomes may contribute to carcinogenesis by the loss of putative tumor suppresser genes [21]. In addition, the presence of additional chromosomal aberrations observed in many of the cases suggests that other genetic alterations have been superimposed on the primary changes to lead to a fully malignant phenotype. Pedersen-Bjergaard et al. suggested the multistep development of t-AML from therapy-related MDS [2]. Primary and early chromosomal abnormalities could be the loss of a whole chromosome 7 or deletion of its long arm, deletions of the long arm of chromosome 5, or a deletion of chromosome band 13q14. Secondary aberrations may have accumulated before the establishment of overt t-AML so that the karyotype of this type of t-AML was complex at the diagnosis of AML. The other type of t-AML, i.e. patients presenting with overt AML, showed cytogenetic divergence; balanced translocations involving the long arm of chromosomes 8, 11, and 21 predominated.

Among the five patients with t-AML in this series, two patients had a history of chemotherapy including alkylating agents and two others had received radiation therapy. Three of these patients showed a complex karyotype with unbalanced aberrations. The close resemblance of chromosomal findings between patients with t-AML and those with AML/TLD or with AML transformed from MDS suggests that the latter patients may have been exposed to chemical agents or radiation [6,22]. The remaining patient, who had received intravenous cyclophosphamide, doxorubicin, vincristine and, subsequently, oral etoposide, developed AML with a t(9;11)(p22;q23). Translocations to chromosome band 11q23 or 21q22 are known to be associated with the treatment with epipodophyllotoxins [5].

TP53 mutation was detected in advanced variants of MDS, AML/TLD, or t-AML. Since it was observed in cases of MDS, *TP53* mutation seems to be an early genetic event [13]. Another patient (case 337) also showed a mutation when peripheral blood cytopenia appearing 6 months before the diagnosis of t-AML was confirmed. However, it was discordant in three other

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Table 6
Correlations between chromosomal findings and point mutations (I)

	Chromosomes			
	normal	balanced	unbalanced	Total
N-*RAS* mutation	0	2	8	10
TP53 mutation	0	4	8	12
Either	0	5* (24%)	15*(44%)	20
None	13	21	19	53
Total	13	26	34	73

n.s. (0 vs. 5*); n.s. (5* vs. 15*); p=0.04 (0 vs. 5*); p=0.01 (0 vs. 15*)

* Includes each patient with both N-a*RAS* and *TP53* mutation.
Statistics were calculated with Fisher's exact probability test with Yate's continuity correction.

patients with AML/TLD or t-AML in which a *TP53* mutation appeared at relapse (cases 603, 605, and 701). Cytogenetically, *TP53* mutations were frequently associated with a complex karyotype; they predominated in patients with chromosome 17 abnormalities, including a 17p monosomy [23]. These observations reflect the accumulation of genetic lesions, or a consequence of dysfunction of the *TP53* gene as a guardian of the genome [24].

N-*RAS* mutation was detected in 29% and 20% of the patients with AML/TLD and AML transformed from MDS, respectively. It was observed that those MDS patients harboring an N-*RAS* mutation were more likely to evolve to AML than patients without the mutation [12,25]. Radich et al. reported that bone marrow cells of AML patients presenting an N-*RAS* mutation revealed dysplastic changes characterizing MDS or AML/TLD, suggestive of an antecedent but clinically undetected MDS phase in these patients [25]. It is postulated that some of the N-*RAS* mutations in p-AML were contributed by patients with clinically undetected antecedent MDS; if so, the actual incidence of N-*RAS* mutations in p-AML might be lower than would be predicted (approximately 30%). We found only one patient with an N-*RAS* mutation in the present p-AML group when patients presenting bone marrow dysplasia were excluded.

G:C to A:T transitions at non-CpG dinucleotides and G:C to T:A transversions are considered to be of exogenous origin, suggestive of exposure to carcinogens [26–30]. Among the 12 mutations of the *TP53* gene, five were G:C to A:T transitions at non-CpG and two were G:C to T:A transversions, suggesting an exogenous origin [26–28]. The mutational spectrum of N-*RAS* mutations suggested an exogenous origin in nine of the present patients: G:C to TA in six and G:C to A:T at non-CpG in three. Since a predominance of a G to A transition at codons 12 and 13 is known, the N-*RAS* mutations revealed here may be indicative of an exposure to environmental alkylating chemicals [29,30]. In addition, the finding of two patients with simultaneous *TP53* and N-*RAS* mutations suggested that these two mutations were both of exogenous origin. Thus, the predominance of exogenous-type mutations in our series of AML, mainly in AML/TLD, t-AML, and AML transformed from MDS, suggests an exposure to known or unknown carcinogens in the patients' home and/work environment.

Consequently, the predominance of balanced chromosomal aberrations and the low incidence of N-*RAS* and *TP53* gene mutations in primary overt AML suggest that the leukemogenic potential is prompted by the chromosomal alterations. Such leukemia may also be induced by agents targeting topo II, presenting a rapid onset of the disease and translocations involving 11q23 or t(8;21), t(15;17), or an inv(16) [6]. The other types of AML, i.e. AML/TLD, AML transformed from MDS, and t-AML were associated with a loss of all or parts of chromosomes 5 or 7 in the majority of patients. The mutational spectra of the N-*RAS* and *TP53* genes suggest that these types of leukemia may have been induced by alkylating chemicals used for a previous malignancy or by occupational or environmental exposure. The finding that patients with p-AML with a normal karyotype did not present any mutation of the N-*RAS* or *TP53* genes suggests that the mechanism of leukemogenesis is due to a submicroscopic balanced chromosomal alteration rather than a submicroscopic deletion, since submicroscopic translocation is well

Table 7
Correlations between chromosomal findings and point mutations (II)

	Number of chromosomal abnormalities*		Chromosomal abnormality with or without deletions**	
	0 - 2	3 or more	with deletion	without deletion
N-*RAS* mutation	5/53 (9%)	5/20 (25%)	4/21(19%)	6/52 (12%)
	n.s.		n.s.	
TP53 mutation	2/53 (4%)	10/20 (50%)	9/21(43%)	3/52 (6%)
	p=0.0001		p=0.0004	

* Total number of chromosomal abnormalities observed in the mainline which were counted as described in the text.
** Deletion includes −5/5q−, −7/7q−, −12/12p−, or −17/17p−.

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defined in Ph-translocation-negative but BCR/ABL gene rearrangement-positive chronic myelogenous leukemia [31]. Alternatively, these AML patients with a normal karyotype may have genetic changes at other crucial genes that were not examined in this study.

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