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Gene mutations in the pathogenesis of t-MDS

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A B S T R A C T

Alternative genetic pathways for t-MDS and t-AML were previously suggested based on characteristic chromosome aberrations identical with those observed in *de novo* MDS and AML. The recurrent balanced translocations and inversions of these diseases in most cases result in chimeric rearrangement and inactivation of genes for hematopoietic transcription factors (class II mutations) which disturb cellular differen-tiation. Recently, activating point mutations or internal tandem duplications of genes for signal transduction in the receptor tyrosine kinase – *RAS/BRAF* pathway (class I mutations) have gained interest in *de novo* MDS and AML. A synergism between class I and class II mutations in the development of AML has been suggested. This hypothesis is now supported by our investigations of 140 unselected patients with t-MDS or t-AML for class I and class II mutations. A clustering of class I mutations in the different genetic pathways support the model for leukemic transformation.

Therapy-related myelodysplasia (t-MDS) and acute myeloid leukemia (t-AML) are the most serious long-term complications of cancer therapy. They offer a unique opportunity to study the etiology of MDS and AML and to study genetic abnormalities related to presentation of the disease as t-MDS, as overt t-AML or to transformation from t-MDS to t-AML.

A highly increased risk of t-MDS and t-AML, in most cases presenting as t-MDS, was first observed after therapy with alkylating agents. All al-kylating agents in clinical use have in well defined cohort studies been demonstrated as leukemogenic. The cumulative risk of t-MDS/t-AML has in intensively treated patients been shown to increase by approxi-mately ¼-1% per year from 2 to 5 years after start of therapy for subsequently to stabilize. The risk of leukemia has been shown to increase markedly by an increasing cumulative dose of alkylating agents and by increasing age of the patients. Schedule for administration of the drugs has not been shown to play a role. Most patients with t-MDS or t-AML after therapy with alkylating agents present the chromosomal defects 5q-/-5 and 7q-/-7 often combined with other abnormalities. Such pa-tients respond very poorly to intensive antileukemic chemotherapy.

Subsequently, also topoisomerase II inhibitors as anthracyclines, epipodophyllotoxin derivatives and mitoxantrone were demonstrated as leukemogenic. These drugs have in most studies until now been administered in combination chemotherapy-regimens often also including an alkylating agent or a platin derivative. The risk of leukemia after therapy with topoisomerase II inhibitors has been in the same order of magnitude as following alkylating agents. Some studies have shown dose de-pendency, others have questioned the importance of dose for the risk. Patient age when treated has not been demonstrated as an independ-ent risk factor, and interestingly many children have recently been observed developing t-AML after topoisomerase II inhibitors. At least for the epipodophyllotoxins, more frequent administration seems to result in a higher risk. The disease after these drugs characteristically present as overt t-AML, often with a latent period of only 1-2 years and with one of the recurrent balanced chromosome translocations known from *de novo* AML. These patients often respond rather favourably to intensive antileukemic chemotherapy.

Whereas the mechanisms leading to the development of the recurrent balanced translocations in t-MDS and t-

Table 1. Gene mutations in 140 patients with t-MDS/t-AML.

	Type of gene	*Gene mutated*	*t-MDS (n=89)*	*t-AML (n=51)*	*Significance*
I	Transcription factors	*AML1*	20	2	*p*=0.003
		CEBPA	0	0	-
	?	*NPM1*	4	7	*p*=0.09
	Receptor tyrosine	*FLT3*	1	10	*p*=0.0002
	kinases	*cKIT*	0	2	NS
		cFMS	0	0	-
		JAK2	2	0	NS
II	Genes more downstreams	*KRAS/NRAS*	7	7	NS
	In the kinase pathway	*BRAF*	0	3	NS
		PTPN11	2	2	NS
III	Other types	*P53*	25	9	*p*=0.22

AML and their genetic results with inactivation of genes for hematopoietic transcription factors (class II mutations) have been investigated in detail during the last decades,[1-5] the mechanisms leading to development of unbalanced aberrations and their genetic effects remain more obscure. These topics will be discussed in more detail later on at this meeting. Based on cytogenetic characteristics we previously suggested different genetic pathways of t-MDS and t-AML.[6,7] These pathways were subse-quently confirmed by different gene expression profiles, primarily for patients belonging to pathway I defined by the chromosomal defects 7q$^{-/-}$7 but normal chromosomes 5, and for patients in pathway II defined by 5q-/-5 with or without 7q-/-7.[8,9]

Aim of the studies

Recently, activating mutations of genes involved in signal transduction and cell proliferation (class I mutations) have gained increasing interest in *de novo* MDS and AML. The genes involved belong primarily to the tyrosine kinase receptors or are genes more downstream in the *RAS/BRAF* pathway. We have now investigated 140 unselected patients with t-MDS (n=89) or t-AML (n=51) previously classified in 8 dif-ferent genetic pathways for mutations of 8 of these genes and for mutations of *AML1*, *p53 CEBPA* and *NPM1* by conventional techniques (Table 1).[10-14] A different clustering of 10 of these mutations in the different pathways was observed supporting the existence of the pathways and a significant association between class I and class II mutations indicating cooperation in leukemogenesis.[15]

Results

Pathway I was originally defined by the abnormalities 7q-/-7, by normal chromosomes 5 and by the absence of recurrent balanced aberrations.[6,7] Patients in this pathway often present a less complicated karyo-type. In our series 35/39 patients in this pathway had received alkylating agents and 35/39 presented as t-MDS. Methylation of the *p15* promotor has previously been demonstrated as a common phenomenon significantly associated with 7q-/-7.[16] Subsequently point mutations of the transcription factor *AML1* have been shown to cluster in patients in pathway I.[11,17] Thus, 15/22 of our cases of t-MDS and t-AML with AML1 point mutations belong to pathway I. Mutations of *AML1* were significantly associated with presentation of the disease as t-MDS and with subsequent progression to overt t-AML.

Pathway II was defined by the abnormalities 5q-/-5 without any of the recurrent balanced chromosome aberrations of *de novo* MDS or AML.[6,7] Patients in this pathway had likewise in most cases previously been treated with alkylating agents (27/34 cases in our series) and presented as t-MDS (26/34 cases in our series). Characteristically they present complex, often very complex, karyotypes, sometimes also including 7q-/-7 as well as chromosome derivatives composed of material from several different chromosomes.[18] They often present point mutation of p53 as observed in 25/34 of our patients in this pathway[9,10,15,] frequently with loss of heterozygosity for the gene, in some cases due to the chromosomal defects 17p-/-17. If going into detail, duplication or low copy number amplification of chromosome bands 11q23 and 21q22 may also be includ-

Table 2. Charateristics of patients with t-MDS/t-AML and a normal karyotype (n=24).

Uncharacteristic cases		16/24
Previously RT only		6/24
Non-leukemogenic drugs only		3/24
Abnormal latent period:	≤24 months	7*/24
	≥96 months	3/24

** only 2 of these received topo II inhibitors.*

Table 3. Gene mutations in patients with t-MDS/t-AML and a normal karyotype (n=24).

Mutations demonstrated	: 17/24		
FLT3 + NPMI	: 4/24	*NRAS*	: 3/24
NPMI	: 3/24	*KRAS*	: 1/24
FLT3	: 2/24	*MLL ITD*	: 1/24
NPMI + NRAS	: 1/24	*AML1*	: 1/24
		p53	: 1/24

ed in the very complex karyotypes.[19,20] Point mutations of AML1, as characteristically observed in pathway I, are not common in this pathway as observed in only 3/34 patients in pathway II.

Pathways III-VI comprise patients with the recurrent balanced chromosome aberrations known from *de novo* MDS and AML, most often reciprocal translocations.[6,7] In *de novo* as in therapy-related AML these abnormalities have been shown to result in chimeric rearrangement of the *MLL* gene at 11q23 (pathway III), rearrangement of the core binding factor genes *AML1* and CBFB at 21q22 and 16q22 (pathway IV), rearrangement of the RARA gene at 17q12-21 (pathway V) or rearrange-ment of the *NUP98* gene at 11p15 (pathway VI). All these genes code for hematopoietic transcription factors. In our series 16/23 patients in these pathways had previously been treated with topoisomerase II in-hibitors and 20/23 presented as overt t-AML. Mutations of genes for signal transduction (class I mutations) clustered in our series differently in these pathways.[12-15] In pathway III, *RAS, BRAF* or *FLT3* mutations were observed in 6/11 patients.[14] In pathway IV, one of our only two pa-tients with mutations of *c-KIT* and two other *PTPN11* mutations were observed and in pathway V one of only two patients presented a *FLT3 ITD*. The very few observations in pathways IV and V, however, are supported by the experience from AML *de novo*. In larger series of pa-tients mutations of c-KIT have been shown as characteristic for patients with rearrangement of the core binding factor genes.[21-23] Similarly, *FLT3 ITD* have been shown to cluster in patients with t(15;17) or a normal karyotype.[24-26]

Pathway VII include patients with t-MDS or t-AML and a normal karyo-type. Newer techniques such as M-FISH have not been able in most of such cases to demonstrate chromosome aberrations overlooked by conventional G-banding.[18] Patients with a normal karyotype most often present as overt t-AML as observed in 15/24 of our cases and fre-quently show uncharacteristic clinical findings for instance occurrence after radiotherapy only (Table 2). Such treatment had previously been administered to 6/25 cases of t-MDS or t-AML with a normal karyotype versus 11/116 cases of t-MDS or t-AML with chromosome abnormalities in our series. Another three patients of 24 patients with a normal karyo-type had received therapy with non-leukemogenic agents as antime-tabolites.

Finally 10/24 patients with a normal karyotype presented with either a very short or a very long latent period from start of treatment, raising doubt about their causal relationship to previous therapy. Interestingly, 17/24 patients with a normal karyotype presented a broad spectrum of gene mutations (Table 3). Most common were *FLT3 ITD* and *RAS* mutations followed by *NPM1* mutations. In this subgroup *FLT3* and *NPM1* mutations were often observed simultaneously in the same patient as previously observed in AML *de novo*.[27-30]

All these findings, including a separate gene expression profile for pa-tients with AML and a normal karyotype,[31] indicate, that this type of patient with t-MDS or t-AML belong to a specific genetic pathway. Their etiology may be different from that of patients in pathway I and II: dam-age by alkylation and from patients in pathways III-VI with illegitimate recombinations related to the activity of topoisomerase II. Although radiotherapy may be responsible for some cases in pathway VII, also other so far unknown types of etiology must be considered.

Pathway VIII comprises patients with *Other Cytogenetic Abnormali-ties* mainly unbalanced aberrations not involving chromosome arms 5q or 7q such as +8, del(11q), abnormalities of 12p and 12q, del(13q) or del(21p). Most patients in this pathway (15/20) presented as t-MDS, and 10/20 patients were clinically *uncharacteristic* cases as observed in pathway VII. In total 6/20 patients in pathway VIII presented gene mutations including two with *RAS* mutations and another two with mutations of *NPM1*.

Gene cooperations

Previously, an association and possible cooperation between muta-tions of genes involved in signal transduction in the tyrosine kinase re-ceptor *RAS-BRAF* pathway (class I mutations) and mutations of genes for hematopoietic transcription factors (class II mutations) was demon-strated.[32,33] If considering the *NPM1* gene as a transcription factor, as it is DNA binding and has not been shown involved in signal transduction, in total 33 of our 140 patients with t-MDS or t-AML presented class I mutations and 58 patients presented class II mutations. An association between class I and class II mutations was observed in 24 patients

($\chi^2 = 15.78$, p=0.0001) Further studies of t-MDS and t-AML and their genetic abnormalities may lead to an increased understanding of the genetic abnormalities involved in the pathogenesis of MDS and AML.

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