Gene mutations in the pathogenesis of t-MDS

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 differentiation. 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.
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 subsequently confirmed by different gene expression profiles, primarily for patients belonging to pathway I defined by the chromosomal defects 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 karyotype. In our series 35/39 patients in this pathway had received alkylating agents and 35/39 presented as t-MDS. Methylation of the \(p15\) promoter 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>
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<tr>
<th>Pathway</th>
<th>Type of gene</th>
<th>t-MDS ((n=89))</th>
<th>t-AML ((n=51))</th>
<th>Significance</th>
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<td>P53</td>
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ed in the very complex karyotypes. 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. 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 rearrangement 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 inhibitors 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. In pathway III, RAS, BRAF or FLT3 mutations were observed in 6/11 patients. In pathway IV, one of our only two patients 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 patients mutations of c-KIT have been shown as characteristic for patients with rearrangement of the core binding factor genes. Similarly, FLT3 ITD have been shown to cluster in patients with t(15;17) or a normal karyotype.

Pathway VII include patients with t-MDS or t-AML and a normal karyotype. Newer techniques such as M-FISH have not been able in most of such cases to demonstrate chromosome aberrations overlooked by conventional G-banding. Patients with a normal karyotype most often present as overt t-AML as observed in 15/24 of our cases and frequently 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 karyotype had received therapy with non-leukemogenic agents as antitabolics.

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.

All these findings, including a separate gene expression profile for patients with AML and a normal karyotype, 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: damage by alkylating agents 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 Abnormalities 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 mutations of genes involved in signal transduction in the tyrosine kinase receptor RAS-BRAF pathway (class I mutations) and mutations of genes for hematopoietic transcription factors (class II mutations) was demonstrated. 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.

References

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17. Andersen MK, Christiansen DH, Kirchhoff M, Pedersen-Bjergaard J. Duplication or amplification of chromosome band 11q23, including the unarranged MLL gene, in a recurrent abnormality in therapy-related MDS and AML and is closely related to mutation of the TP53 gene and to previous therapy with alkylating agents. Genes, Chromosomes & Cancer 2001;31:53-41.


