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Chromosome and Molecular Abnormalities in Myelodysplastic Syndromes

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Abstract

Cytogenetic abnormalities are seen in approximately 50% of cases of myelodysplastic syndrome (MDS) and 80% of cases of secondary MDS (following chemotherapy or radiotherapy). These abnormalities generally consist of partial or complete chromosome deletion or addition (del5q, –7, +8, –Y, del20q), whereas balanced or unbalanced translocations are rarely found in MDS. Fluorescence hybridization techniques (fluorescence in situ hybridization [FISH], multiplex FISH, and spectral karyotyping) are useful in detecting chromosomal anomalies in cases in which few mitoses are obtained or rearrangements are complex. Ras mutations are the molecular abnormalities most frequently found in MDS, followed by p15 gene hypermethylation, FLT3 duplications, and p53 mutations, but none of these abnormalities are specific for MDS. The rare cases of balanced translocations in MDS have allowed the identification of genes whose rearrangements appear to play a role in the pathogenesis of some cases of MDS. These genes include MDS1-EVI1 in t(3;3) or t(3;21) translocations, TEL in t(5;12), HIP1 in t(5;7), MLF1 in t(3;5), and MEL1 in t(1;3). Genes more frequently implicated in the pathogenesis of MDS cases, such as those involving del5q, remain unknown, although some candidate genes are currently being studied. Cytogenetic and known molecular abnormalities generally carry a poor prognosis in MDS and can be incorporated into prognostic scoring systems such as the International Prognostic Scoring System. *Int J Hematol.* 2001;73:429-437.

Key words: Myelodysplastic syndromes; Chromosomes; Gene rearrangements

1. Introduction

Myelodysplastic syndromes (MDS) are clonal stem cell disorders characterized by ineffective hematopoiesis and a high risk of progression to acute myeloid leukemia (AML) [1]. The etiology of MDS is generally unknown, but some cases of MDS (secondary MDS) can occur after the use of antineoplastic agents (mainly alkylating agents) or after exposure to benzene derivatives [2-4].

Cytogenetic abnormalities can be found in approximately one half of the cases of MDS by using conventional banding techniques, and these abnormalities carry strong prognostic value [5-13]. Other cytogenetic techniques, including fluorescence in situ hybridization (FISH), multiplex FISH (M-FISH), and spectral karyotyping (SKY) can give further information on chromosomal rearrangements [14-17].

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Due largely to the rare occurrence of balanced translocations in MDS (as opposed to AML), specific gene abnormalities playing a role in the myelodysplastic process have been difficult to identify in myelodysplastic disorders. Some gene rearrangements that can be observed in other myeloid malignancies and are therefore not specific to MDS, are, however, observed in a substantial percentage of MDS cases.

2. Cytogenetic Abnormalities

2.1. Chromosomal Abnormalities Observed in MDS by Using Conventional Cytogenetic Analysis

The primary cytogenetic abnormalities observed in MDS are shown in Table 1 [5-13]. The incidence of abnormal karyotypes is higher (80%) in secondary MDS. The most frequent rearrangements are partial and complete chromosome loss (especially del5q, –7, –Y, and del20q) or chromosome gain (most frequently +8), whereas balanced translocations are rare. Unbalanced chromosomal translocations are somewhat more frequent, leading to partial monosomy or trisomy [ie, 17p monosomy in t(5;17) or 7q monosomy in t(1;7)]. None of

Table 1.
Main Cytogenetic Abnormalities Encountered in Myelodysplastic Syndromes (MDS)

Abnormality	Approximate Incidence, %	
	De Novo MDS	Secondary MDS
Partial chromosomal deletion		
del5q	20	20
del20q	3 to 4	<1
del7q	1 to 2	10
der or del11q	2 to 3	<1
der or del12p	1 to 2	3 to 4
del13q	1	<1
Chromosome loss		
monosomy 7	10 to 15	50
loss of Y chromosome	3 to 4	10
monosomy 17	3	5 to 7
Chromosome gain		
trisomy 8	10 to 15	10
trisomy 11	3	1
trisomy 21	2	1
Translocations (balanced or unbalanced)		
t(3;3)(q21;q26)	1 to 2	3
t(1;7)(p11;p11)	<1	4 to 5
t(5;17)(p11;p11)	1 to 2	4 to 5
t(3;5)(q25.1;q34)	<1	2 to 3
t(5;7)(q11;p11)	<1	2
t(11p15)	<1	
t(1;3)(p36;q21)	<1	
Other findings		
iso(17q)	<1	3 to 4
inv(3)(q21q26)	<1	3
Complex findings (≥3 chromosome abnormalities)	15 to 20	50

the rearrangements described in Table 1 are specific to MDS; they can also be observed in AML or in chronic myeloproliferative disorders, but their relative incidence is characteristic of MDS. Complex cytogenetic abnormalities (at least 3 chromosomal abnormalities) are particularly frequent in secondary MDS. Balanced translocations, including t(15;17), inv(16), t(8;21), and t(9;11), are not found in MDS but are specific to AML. Some of the patients with these translocations may occasionally present with less than 30% or even 20% marrow blasts, but there is an agreement that they should be classified as AML rather than MDS.

2.2. FISH, M-FISH, and SKY Analysis of Chromosomes in MDS

FISH analysis has proven useful in the detection of chromosomal abnormalities when cytogenetic analysis yielded few analyzable mitoses, especially when centromeric probes for chromosomes 7 and 8 are used for the detection of monosomy 7 and trisomy 8, respectively. These probes are also useful in confirming the clonality of the abnormality by examining a large number of cells when only 1 or 2 mitoses with –7 or +8 are seen by conventional cytogenetic analysis. FISH analysis can be combined with immunophenotyping (FICTION method), allowing the determination of the clonality of different cell populations (in our experience with –7 and +8, only myeloid cells, but not lymphoid cells, carried the anomaly) [18].

SKY and M-FISH are methods that, by combining different fluorochromes, allow each of the 23 chromosome pairs to be stained with a different color. These methods are especially useful in identifying complex translocations. Kakazu et al [16] used SKY to study 20 cases of MDS, many of which involved complex abnormalities found using conventional cytogenetic analysis. SKY was used to identify the chromosomal basis of 38 mar, add, and hsr; correct the misidentification by G banding of 8 abnormalities; and reveal 6 cryptic translocations in 5 cases. Furthermore, in 3 of 11 cases with –5/del5q and 4 of 8 cases with –7/del7q, lost material was detected by SKY in unbalanced translocations. Sixty chromosome losses were identified by G banding in 16 cases with multiple chromosome abnormalities. SKY showed that, in 26 (43%) of these cases, losses were not complete but that chromosome fragments had been translocated to a variety of partner chromosomes. Another study, analyzing by FISH several cases of 5q or 7q deletion, also confirmed that lost material was often translocated to other chromosomes, sometimes after chromosome 5 or 7 breakage into more than 2 segments (chromosome fragmentation), giving rise to marker chromosomes [19].

2.3. Prognostic Value of Cytogenetic Findings in MDS

Cytogenetic findings have strong prognostic value in MDS [8,20,21]. Presence of an abnormal clone is generally a poor prognostic factor, with few exceptions. An international workshop based on data from more than 900 patients found normal karyotype, isolated del5q, del20q, and loss of Y chromosome to be factors associated with relatively favorable prognoses; monosomy 7 (or del7q) and complex cytogenetic findings to be associated with poor prognoses; and other findings to have intermediate prognostic significance (Table 2) [20].

The prognostic value of karyotypes appears to be independent of that of other main prognostic parameters, including the percentage of marrow blasts and cytopenias. This characteristic has allowed the design of prognostic scoring systems that incorporate cytogenetic findings, especially the International Prognostic Scoring System based on results of the international workshop (Table 3) [20].

2.4. Chromosome Findings and Etiology of MDS

A high incidence of –5/del5q and/or –7/del7q is seen in cases of MDS occurring after exposure to alkylating agents or benzene derivatives [22]. In vitro, the benzene derivative metabolites hydroquinone and catechol were shown to act in synergy to induce dose-dependent hypodiploidy and 5q31 deletion in a human cell line [23]. In addition, hydroquinone can selectively induce del5q31 and –7 in human $CD34^+CD19^-$ bone marrow cells [24]. MDS occurring after exposure to alkylating agents are also characterized by an increased incidence of dicentric chromosomes compared with the incidence in de novo MDS, suggesting a specific susceptibility to chromosome breakage at the centromere [25].

Table 2.
International Prognostic Scoring System for Myelodysplastic Syndromes: Survival and Acute Myeloid Leukemia Evolution

	Score Value*				
Prognostic Variable	0	0.5	1.0	1.5	2.0
Bone marrow blasts, %	<5	5-10		11-20	21-30
Karyotype†	Good	Intermediate	Poor		
Cytopenic episodes	0-1	2-3			

*Scores for risk groups are as follows: Low, 0; Intermediate-1, 0.5-1.0; Intermediate-2, 1.5-2.0; and High ≥2.5.

†Good indicates normal, –Y, del(5q), del(20q); Poor indicates complex (≥3 chromosome abnormalities) or chromosome 7 anomalies; Intermediate indicates other abnormalities.

Trisomy 8 occurring in MDS and AML, on the other hand, has been associated with tobacco smoking in some studies [26]. Other studies have indeed shown that smoking significantly increased the risk of MDS (and AML) [27].

West et al [22], in a large case-control study of environmental and occupational exposures to more than 90 potential hazards, found a higher incidence of cytogenetic rearrangements (especially of chromosomes 5 and/or 7) in exposed versus nonexposed MDS cases. Exposure to radiation was associated with abnormalities of chromosome 8.

A study of 12 patients who developed MDS after autologous stem cell transplantation showed, in 9 patients, that the chromosome abnormalities observed at diagnosis of MDS were already present before conditioning chemotherapy in a small proportion of cells [28]. This observation confirmed that the stem cell damage leading to MDS resulted more from prior conventional chemotherapy than from the conditioning regimen itself. Finally, a possible correlation between –7 or del7q and previous exposure to ionizing radiation has recently been suggested [29].

3. Molecular Abnormalities in MDS

3.1. Description of Molecular Abnormalities Found in MDS

The low incidence of balanced translocations in MDS probably explains to some extent the small number of genes involved in the pathogenesis of the MDS disorders discovered so far. In addition, none of the genes involved in these balanced translocations are specifically involved in MDS.

3.1.1. Mutations of RAS and NF1 genes

The RAS family of proto-oncogenes encodes proteins that regulate cellular proliferation and differentiation by cycling between an active guanosine triphosphate (GTP)-bound state and an inactive guanosine diphosphate (GDP)-bound state. Neurofibromin, the 327-kd protein encoded by the gene for neurofibromatosis type 1 (NF1), contains a domain with considerable sequence homology with both yeast and mammalian GTPase-activating proteins. When this GTPase-activating domain of neurofibromin binds Ras protein, it accelerates the conversion of Ras-GTP to Ras-GDP by increasing intrinsic Ras-GTPase activity.

Point mutations involving codons 12, 13, or 61 of members of the RAS gene family (mainly N-RAS, less often K-RAS, and rarely H-RAS), leading to an activated RAS protein, certainly constitute the most frequent molecular anomaly identified in MDS [30]. Incidence of RAS mutations of approximately 30% in MDS was reported in early series, but these mutations often occurred in patients with advanced MDS. The incidence of RAS mutations at diagnosis in MDS could instead be in the range of 10% to 15%, with a higher incidence in chronic myelomonocytic leukemia (CMML) compared with other MDS subgroups. Whether RAS mutations are early or late events in MDS is controversial: RAS mutations often appear during the disease course, as shown in some longitudinal studies; in other cases, RAS mutations appeared to be earlier events in the pathogenesis of MDS. RAS mutations are usually detected by DNA amplification of exon 1 (codons 12 and 13) and exon 2 (codon 61) of RAS genes followed by hybridization with oligonucleotide probes specific for each possible mutation (dot blot technique).

The risk of malignant myeloid disorders, especially in MDS occurring in young children with NF1, is 200 to 500 times the normal risk. Cases of MDS occurring in children with NF1 are associated with NF1 gene deletions and with a decrease in neurofibromin, the product of the NF1 gene [31]. Recently, inactivation of both NF1 alleles was found in most children with NF1 and MDS [32]. Mutations of the NF1 gene were also observed in MDS in children without clinical evidence of NF1 [33].

Inactivation of neurofibromin, like RAS gene mutations, leads to RAS activation [34]. This finding suggests the importance of a deregulated RAS pathway in the pathogenesis of MDS. The importance of this pathway is further substantiated by the fact that RAS mutations often occur in the absence of chromosomal abnormalities, suggesting the involvement of a limited number of genetic defects in pathogenesis. On the other hand, no abnormalities of the NF1 gene have been found in adult MDS [35].

3.1.2. Mutations of the p53 Gene and Other Tumor Suppressor Genes

3.1.2.1. p53 Gene Mutations

p53 gene mutations occur in approximately 5% of MDS cases [36,37]. As in other malignancies, p53 gene mutations occur predominantly in exons 5 to 8 of the gene and are generally missense mutations inactivating p53. p53 gene mutations can be detected by single-strand conformation–polymorphism analysis of exons 5 to 8 of the gene or immunocytochemical analysis of bone marrow slides (which detects p53 overexpression, almost synonymous

Table 3.
Proposed World Health Organization Classification of Myeloid Neoplasms*

Myeloproliferative diseases
Chronic myelogenous leukemia, Philadelphia chromosome positive [t(9;22)(qq34;q11), *BCR/ABL*]
Chronic neutrophilic leukemia
Chronic eosinophilic leukemia/hypereosinophilic syndrome
Chronic idiopathic myelofibrosis
Polycythemia vera
Essential thrombocythemia
Myeloproliferative disease, unclassifiable
Myelodysplastic/myeloproliferative diseases
Chronic myelomonocytic leukemia
Atypical chronic myelogenous leukemia
Juvenile myelomonocytic leukemia
Myelodysplastic syndromes
Refractory anemia
With ringed sideroblasts
Without ringed sideroblasts
Refractory cytopenia (myelodysplastic syndrome) with multilineage dysplasia
Refractory anemia (myelodysplastic syndrome) with excess blasts
5q– syndrome
Myelodysplastic syndrome, unclassifiable
Acute myeloid leukemias†
AML with recurrent cytogenetic translocations
AML with t(8;21)(q22;q22), AML1(CBF-α)/ETO
Acute promyelocytic leukemia [AML with t(15;17)(q22;q11-12) and variants, PML/RAR-α]
AML with abnormal bone marrow eosinophils [inv(16)(p13q22) or t(16;16)(p13;q11), CBFβ/MYH11X]
AML with 11q23 (MLL) abnormalities
AML with multilineage dysplasia
With prior myelodysplastic syndrome
Without prior myelodysplastic syndrome
AML and myelodysplastic syndromes, therapy-related
Alkylating agent–related
Epipodophyllotoxin-related (some may be lymphoid)
Other types
AML not otherwise categorized
AML minimally differentiated
AML without maturation
AML with maturation
Acute myelomonocytic leukemia
Acute monocytic leukemia
Acute erythroid leukemia
Acute megakaryocytic leukemia
Acute basophilic leukemia
Acute panmyelosis with myelofibrosis
Acute biphenotypic leukemias

*Only major disease categories are listed; subtypes and variants to be discussed in detail in a future publication by the World Health Organization. AML indicates acute myeloid leukemia; CBF, core-binding factor; PML, progressive multifocal leukoencephalopathy; RAR, retinoic acid receptor; MLL, mixed-lineage leukemia.

†Acute lymphoid leukemias are included under lymphoid neoplasms.

with p53 missense mutation). p53 mutations are almost exclusively seen in refractory anemia with excess of blasts, refractory anemia with excess of blasts in transformation, and CMML and are generally associated with deletion of the nonmutated allele, through a chromosome 17p deletion [resulting from t(5;17) or t(7;17), unbalanced translocations, or monosomy 17].

p53 mutations are also associated with a typical form of dysgranulopoiesis combining pseudo–Pelger-Huet hypolobulation and vacuoles in neutrophils, which in our experience and that of other groups is almost never seen in MDS without a 17p deletion. Thus, MDS with a 17p deletion appears to constitute a specific morphological-cytogenetic and molecular entity, the 17p– syndrome [38,39].

Most MDS cases with p53 mutations have complex cytogenetic abnormalities [38]. It is unknown whether complex cytogenetic abnormalities are secondary to the p53 mutation, because p53 inactivation can lead to chromosomal instability. In our experience, p53 mutations in MDS (and AML) were always found at diagnosis and were not acquired during the disease course. However, this does not imply that they constitute an early event in pathogenesis. Furthermore, because they are generally associated with complex chromosomal abnormalities, and therefore with many genetic defects, the precise role of p53 mutations in the pathogenetics of MDS is still unknown.

3.1.2.2. p15 and p16 Gene Mutations

p15 and p16 are cycline-dependent kinase inhibitors whose inactivation by deletion, point mutation, or methylation plays a role in carcinogenesis. Inactivation of the p16 gene has very rarely been found in MDS [40]. However, methylation of the p15 gene, in our experience, is frequent and almost constant in MDS, showing progression (with ≥ 10% marrow blasts) suggesting that p15 inactivation is an important step in the progression of MDS [41,42].

3.1.2.3. Retinoblastoma Gene Mutation

Inactivation of the retinoblastoma gene is not seen or is a very rare event in MDS [43].

3.1.3. Genetic Abnormalities Observed in Balanced Translocations in MDS

Rare cases of balanced translocations in MDS have often resulted in the identification of fusion genes between a known gene and a new gene of interest, which could be subsequently cloned.

3.1.3.1. MDS With t(5;12)(q31;p13)

A few cases of MDS, generally having features of atypical CMML with eosinophilia in particular, have been reported [44]. Cloning of the translocation breakpoint allowed the identification in 12p13 of the TEL gene, a member of the ETS gene family. In this translocation, the TEL gene was fused to the platelet-derived growth factor (PDGF)-receptor gene in 5q31. Involvement of the TEL gene in other translocations and/or TEL gene deletions has also subsequently been found in AML and, with a high incidence, in childhood acute lymphoblastic leukemia (ALL). However, TEL gene involvement seems to be very rare in MDS except in the rare cases with t(5;12). 12p is involved in other rearrangements in

MDS through del12p, translocations, or dicentric chromosomes involving 12p (especially with chromosome 13) [45].

3.1.3.2. MDS With t(3;21)(q26;q22), t(3;3)(q21;q26), and inv(3)(q21;q26)

A small number of cases of MDS with t(3;21), generally occurring in patients after treatment with antineoplastic drugs, has been reported. In these cases, the AML_1 gene situated in 21q22 is fused to 1 of the following genes identified in 3q26: EVI1, MDS 1, or both (AML–MDS1-EVI1–complex fusion) or EAP. EVI1, in particular, is activated in the translocation [46,47].

EVI1 also exists as a longer protein, MDS1/EVI1, that includes 188 additional amino acids at the N-terminus. The genes encoding both proteins are expressed at very low levels in the normal bone marrow. The genomic region between the first coding exon of MDS/EVI1 and the first coding exon of EVI1 is 150-300 kb. The majority of the chromosomal breakpoints at the 5′ end of EVI1 in the t(3;3) resulting in EVI1 activation have been mapped in this region. As a consequence of the t(3;3), the cell would be unable to express MDS1/EVI1, although it would express EVI1. MDS/EVI1 is a strong activator of promoters containing the AGATA motif, whereas EVI1 is a repressor. In addition, EVI1 represses activation by the GATA-1 erythroid factor and MDS/EVI1 does not, and MDS/EVI1 is itself repressed by EVI1. Thus, rearrangements at 3q26 involving EVI1 could result in leukemia by a 2-step process involving transcriptional disruption of MDS/EVI1 followed by inappropriate activation of expression of EVI1.

It has also recently been shown that MDS/EVI1 enhanced the growth-inhibitory effect of transforming growth factor (TGF)β, whereas the AML1/MDS1/EVI1 fusion product abrogated growth inhibition in response to TGFβ [48]. Forced expression of the EVI1 gene in embryonic stem cells has been found to increase cell growth, especially the number of megakaryocytic colonies [49].

3.1.3.3. MDS With 11p15 Translocation

Several cases of MDS with balanced translocations involving 11p15 and another chromosome, such as t(11;17)(p15;q21), t(11;12)(p15;q13), and t(7;11)(p15;p15), have been reported, mainly in children and in therapy-related MDS. In all analyzed cases, the NUP98 gene in 11p15 was fused to another gene on the partner chromosome, leading to a fusion transcript [50,51].

3.1.3.4. Other Balanced Translocations in MDS

Rare cases of t(1;3)(p36;q21) have been reported in MDS. Their molecular analysis led to the identification of a new gene in 1p36.3, MEL1, which is highly homologous to the MDS1/EVI1 gene. In this translocation, MEL1 is trancriptionally activated by the ribophorin I gene situated in the 3q21 region, as MDS1/EVI1 in inv3 (q21;q26) or t(3;3)(q21;q26) [52].

The balanced translocation t(3;5)(q25.1;q34) has also been reported in a few cases of MDS with excess of marrow blasts and frequent progression to AML. This translocation leads to chimeric transcripts between the nucleophosmin (NPM) gene in 5q34 (also involved in lymphoma) and a novel gene, myelodysplasia/myeloid leukemia factor 1 (MLF1). MLF1 is normally located in the cytoplasm, whereas NPM-MLF1 is targeted to the nucleus, with highest levels in the nucleolus. Transgenic mice expressing the fusion protein have high levels of myeloid precursors, whereas K562 cell line transfected with the fusion protein undergoes apoptosis [53,54].

Finally, rare cases of MDS with t(5;7)(q33;q11.2) have been reported. This translocation leads to a fusion between the PDGFβ gene and Huntingtin interacting protein 1 (HIP1), located in 17q11.2 [55], leading to activation of PDGFβR tyrosine kinase.

3.1.4. Other Molecular Abnormalities in MDS

3.1.4.1. FMS Mutations in MDS

The FMS gene encodes for the macrophage-colony–stimulating factor (M-CSF) receptor. This gene is found mutated in approximately 10% of MDS cases, with a predominance in CMML, as in RAS mutations [56]. However, most mutations involve codon 969, a mutation that does not lead to activation of the M-CSF receptor. Some mutations involve codon 301, leading to activation of the receptor, but they account for only 10% to 15% of FMS gene mutations. Therefore, the role of FMS gene mutations in MDS still has to be more precisely established.

3.1.4.2. FLT3 Duplication in MDS

Internal tandem duplication within exons 11 and 12 of the FLT3 gene, a subclass III tyrosine kinase receptor, has been reported in approximately 10% of MDS cases, and is associated with a high risk of progression to AML [57].

3.1.4.3. Wilms Tumor Gene Expression

Wilms tumor gene expression in bone marrow cells appears to be correlated with disease progression in MDS [58].

3.1.4.4. Mutations of the AML1 Gene in MDS

The AML1 gene, situated in 21q22, is often disrupted in AML, mainly through t(8;21); in ALL, through t(12;21); and rarely, as seen above, in MDS with t(3;21). Point mutations of the AML1 gene, which inactivated the AML1 protein, have recently been reported in 2 of 37 analyzed cases of MDS. Both mutations resulted in AML1 protein inactivation, in at least 1 case through a dominant-negative effect [59].

3.1.5. Unidentified Genes: From Cytogenetics to Molecular Biology of Specific "Entities" in MDS

In AML, specific morphological-cytogenetic entities such as M_2 AML with t(8;21), M_4 eosino AML with inv(16), and M_3 AML with t(15;17) have been described. As in AML, specific entities with morphological-cytogenetic characteristics

have been identified in MDS, including the 5q– syndrome and, as seen above, the 17p– syndrome. One of these entities, the 5q– syndrome, is taken into account in the new World Health Organization classification of MDS (Table 3) [60]. In AML, specific gene abnormalities are being found in a growing number of these disease entities. This search for specific gene abnormalities has been more elusive in MDS, due largely to the fact that translocations, which offer a good opportunity to identify new genes, are so rare in those disorders. Because chromosomal deletions are frequent in MDS, the role of inactivation of tumor suppressor gene(s) situated on deleted chromosomal fragments in the pathogenesis of MDS is strongly suspected. However, discovering new genes whose abnormalities could specifically be involved in MDS in those large chromosome segments has so far proved difficult, in spite of cytogenetic, FISH, and molecular analysis techniques.

3.1.5.1. del5q and the 5q– Syndrome

del5q can be the sole anomaly in MDS or be part of complex cytogenetic abnormalities. Isolated del5q usually occurs in elderly women, is generally associated with refractory anemia without excess of blasts, macrocytic anemia, thrombocytosis, and typical mononucleated megakaryocytes, features characterizing the 5q– syndrome [61]. In MDS with del5q, the deletion is always interstitial but is of variable extent. A commonly deleted segment involving 5q31 and 5q32 bands was detected using cytogenetic analysis in 91 of 93 analyzed cases of del5q [62]. As a next step, known genes located in this region were analyzed. However, none of the genes coding for growth factors or growth factor receptors situated in this region were found to be part of the commonly deleted segment. A third step was to identify new genes in this segment, of which 1 allele was deleted through del5q and the other allele could be potentially inactivated on the remaining chromosome 5 (by deletion or mutation). By positional FISH and molecular biology techniques, several genes, including IRF_1, EGR_1, C_5 orf3, C_5 orf4, HSPA9, and POP_2, and several chromosomal loci, including D_5S_{89}, D_5S_{413}, and D_5S_{487}, were shown to play possible roles, but no specific candidate has been selected by a consensus among scientists [63-65]. In fact, recent findings suggest the involvement of several critical regions of chromosome 5 in MDS with del5q, and therefore several different genes may play a role. The 5q32 region could be especially involved in the 5q– syndrome, and 5q31 and 5q13.1 bands could be involved in other MDS with del5q [66,67]. Another possibility is that loss of 1 allele of 1 or several genes could lead to impaired hematopoiesis (haploinsufficiency).

3.1.5.2. 17p– Syndrome

As seen above, a strong correlation has been found in MDS between cytogenetic rearrangements leading to a 17p deletion, p53 mutation, and a typical form of dysgranulopoiesis (the 17p– syndrome). In these cases, the extent of the 17p deletion is variable but it always includes deletion of the nonmutated allele. Whether p53 inactivation or inactivation of another gene(s) located in 17p plays a more important pathogenetic role in the 17p– syndrome remains unknown [68,69].

3.1.5.3. Isolated Isochromosome 17q in MDS

Although isolated isochromosome 17q also leads to 17p deletion, patients with this chromosome rearrangement seem to have somewhat different features from patients with with 17p– syndrome, except for the presence of neutrophil hyposegmentation and poor prognosis. The disease in patients with isolated isochromosome 17q often appears to have both myelodysplastic and myeloproliferative features, whereas prominence of the monocyte/macrophage lineage and p53 mutation is infrequent [70].

3.1.5.4. Monosomy 7, del7q, and del20q

Several groups are looking for tumor suppressor genes on the chromosomes involved in monosomy 7, del7q, and del20q, but no candidate gene has been identified so far. Common deleted segments have been identified in 20q11, 7q32-34, and 7q22, however [24,25], and their identification constitutes the first step in the search for candidate genes.

3.2. Molecular Abnormalities Related to MDS Predisposition

The role of several chemical compounds in the pathogenesis of MDS including alkylating agents, benzene and its derivatives, and probably other chemicals used in industry and agriculture is well documented. Several enzymatic systems can be involved in the detoxification or activation of such substances, and glutathione S-transferase (GST) enzymes are among them.

An increased incidence of GST theta 1 null genotype in MDS patients compared to control subjects was found in one study, but not confirmed in 2 other reports [71,72]. We also found, in patients with MDS, a higher incidence of previous exposure to chemicals in patients with normal GST theta 1 genes, suggesting that this gene could have an activating role for some chemical carcinogens [71]. Subjects with both high activity of the CYP2E1 gene and low activity of the NQO1 gene, 2 genes involved in the activation and detoxification, respectively, of benzene and its derivatives, were found to be at increased risk of MDS after exposure to benzene [4]. Future study of other enzymatic systems may help to determine the risk of MDS and AML after certain exposures in a given person and lead to the discovery of effective preventive measures.

3.3. Mitochondrial DNA mutations in MDS

Recently, point mutations in mitochondrial DNA coding for cytochrome c oxidase or cytochrome b have been reported. Such mutations lead to intramitochondrial accumulation of iron in the ferric form (Fe^{3+}) that cannot be used by the enzyme ferrochelatase for the last step of heme synthesis. Such gene defects probably contribute to the pathogenesis of acquired idiopathic sideroblastic anemia [73].

3.4. Telomeres and MDS

At least 2 research groups have shown telomere shortening in approximately 40% of MDS cases. Telomere shortening was correlated with poor prognostic features, including increased percentages of marrow blasts and cytogenetic abnormalities [74,75].

3.5. Molecular Abnormalities and Prognosis in MDS

Ras mutations have been associated with poor response to treatment and poor prognosis in MDS in most, although not all, studies [30]. p53 mutations in MDS are associated with very low response rates to any form of chemotherapy and very poor survival rates [76]. FLT3 duplication is also associated with poorer prognosis [57]. So far, however, no study has clearly determined whether the poor prognosis associated with these molecular abnormalities was independent from that of other main prognostic factors, including bone marrow blast percentage and karyotype.

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