THE MOLECULAR BASIS OF MYELODYSPLASTIC SYNDROMES

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ABSTRACT

Background and Objective. The myelodysplastic syndromes comprise a heterogeneous group of neoplastic disorders characterized by ineffective hematopoiesis with an increased tendency to evolve to acute leukemia. Clinically, the common manifestations include peripheral blood cytopenias of one or more lineages and a normal to hyperplastic bone marrow. MDS has been defined on the basis of morphological criteria, namely the percentage of blast cells in the bone marrow, by the French-American-British study group. Scoring systems have been developed to include such factors as hemoglobin, leukocyte count and age in the evaluation of MDS prognosis. Although useful in the prediction of clinical course and design of therapy regimens, our understanding of the basis of MDS has come from recent advances in molecular analysis of these disorders. This review describes some of the established and recent contributions to our understanding of the molecular basis of the myelodysplastic syndromes.

Evidence and Information Sources. The authors of the present review have been working in the field of myelodysplastic syndromes for several years and have contributed original papers on the molecular pathogenesis of these disorders. In addition, in the present review they have critically examined articles and abstracts published in journals covered by the Science Citation Index® and Medline®.

State of Art and Perspectives. Cytogenetic anomalies and proto-oncogene abnormalities point to new understanding of the pathogenesis of MDS as a sequence of DNA lesions leading to the evolution of the pre-malignant clone. The prognostic significance of these factors in predicting leukemic transformation and survival remains controversial. Characterization of MDS cells in vitro in response to combinations of exogenous growth factors have not only provided valuable information regarding ineffective hematopoiesis in MDS but have provided a new insight into treatment of MDS. One major development in our understanding of MDS is the possible explanation for the apparent paradox of a cellular marrow in combination with peripheral cytopenias. Extensive premature programmed cell death or apoptosis has been reported to be at least partly responsible. It will remain to be seen whether this fundamental characteristic of myelodysplastic hematopoiesis will play a central role in the drug or genetic based therapy in the myelodysplastic syndromes.

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Molecular abnormalities in myelodysplastic syndromes

Studies in MDS have focused on the two classes of genes involved in malignancy, the proto-oncogenes and the tumor-suppressor genes. Activation of proto-oncogenes have been reported to occur by chromosomal translocations, point mutations or gene fusions to create novel oncogenes. Tumor suppressor genes are mostly involved in solid tumors; however, p53 and neurofibromatosis type 1 (NF-1) genes have been reported to be involved in MDS pathogenesis. The NF-1 gene is activated through rearrangements and/or mutation in the genetic disorder Von Recklinghausen’s neurofibromatosis (NF-1 disease) \(^8\). The NF-1 gene product is a member of the family of guanine triphosphatase (GTPase)-activating proteins, which promote hydrolysis of GTP to GDP on the RAS-related GTP-binding proteins. \(^9\) Rearrangements and mutations of the NF-1 gene have been reported in cases of sporadic tumors and in some cases of MDS, either point mutation \(^10\) or allelic loss, in childhood MDS. \(^11\) Abnormalities of the NF-1 gene may therefore be important in the pathogenesis of childhood MDS. Indeed, a high incidence of MDS and AML is seen in children with NF-1 disease. \(^12\) Another study investigated structural rearrangements including rearrangements and deletions by Southern blot analyses using cDNA probes covering the whole coding region, in 35 cases of adult MDS and 8 cases of AML. \(^13\) In this study, although allelic loss of the NF-1 gene was seen in 14 patients, neither rearrangement nor deletion was found in any patient, suggesting that gross abnormalities of the NF-1 gene are rare in adult MDS and AML. This study did not address the possible existence of more subtle abnormalities such as point mutations within the coding region of the NF-1 gene. The exact role of NF-1 aberrations in the pathogenesis of MDS remains to be resolved. The more recently characterized IRF-1 gene, encoding interferon regulatory factor-1, maps to 5q31.1 and its gene product has anti-oncogenic potential. Loss of the IRF-1 gene at one or both alleles has been demonstrated in MDS and AML \(^14\). In one study, however, two

Table 1. French-American-British (FAB) classification of the myelodysplastic syndromes.

<table>
<thead>
<tr>
<th>Category</th>
<th>Characteristics</th>
<th>Mean Survival</th>
</tr>
</thead>
<tbody>
<tr>
<td>Refractory anemia (RA)</td>
<td>Refractory cytopenias</td>
<td>35 months</td>
</tr>
<tr>
<td></td>
<td>Marrow blasts &lt; 5%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ring sideroblasts &lt; 15%</td>
<td></td>
</tr>
<tr>
<td>RA with ringed sideroblasts</td>
<td>Refractory cytopenias</td>
<td>35 months</td>
</tr>
<tr>
<td>(RARS)</td>
<td>Marrow blasts &lt; 5%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ring sideroblasts &lt; 15%</td>
<td></td>
</tr>
<tr>
<td>Chronic myelomonocytic</td>
<td>Marrow blasts &lt; 20%</td>
<td>12 months</td>
</tr>
<tr>
<td>leukemia (CMML)</td>
<td>Monocytes &gt; 1×10⁹/L</td>
<td></td>
</tr>
<tr>
<td>RA with excess of blasts (RAEB)</td>
<td>Refractory cytopenias</td>
<td>18 months</td>
</tr>
<tr>
<td></td>
<td>Marrow blasts 5-20%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ring sideroblasts &lt; 15%</td>
<td></td>
</tr>
<tr>
<td>RAEB in transformation</td>
<td>Refractory cytopenias</td>
<td>6 months</td>
</tr>
<tr>
<td>(RAEB-t)</td>
<td>Marrow blasts 20-30% or Auer rods in blasts</td>
<td></td>
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MDS patients with 5q- syndrome retained both copies of the gene, suggesting that loss of IRF-1 allele(s) is not solely responsible for the development of 5q- syndrome. The retinoblastoma-susceptibility (Rb) gene is an anti-oncogene that frequently harbors alterations in retinoblastomas, sarcomas and some epithelial tumors. Abnormal expression of the Rb protein has been found in 20-30% of AML cases, particularly in M4 and M5 subtypes. Structural alterations of the Rb gene or abnormal expression of the protein, although detected in some cases, appear to be rare in MDS.

Cellular proto-oncogenes can acquire oncogenic potential usually by DNA point mutations as in RAS genes, or by gene deletions as in SRC and ERB. When these mutations introduce alterations in functionally critical domains of the protein, constitutive activity and release from normal regulatory controls in the cell are conferred. Many of the proto-oncogenes discovered to date play a role in normal cellular functions such as cell growth and differentiation.

One gene family that continues to demand attention in the study of MDS is the RAS gene family. The three RAS genes encode highly related 21kD proteins that have been shown to play a central role in the transduction of growth and differentiation factor stimuli. Activation of the RAS gene product, p21RAS, results from exchange of bound GDP for GTP, generating a downstream signal through interaction with effector molecules. Common mutations of the RAS genes are found at codons 12, 13 and 61 and give rise to p21RAS, which is constitutively locked in the active GTP-bound form. These mutations cause inhibition of the hydrolysis of bound GTP or increased rate of guanine nucleotide exchange. Oncogenic activation of one or more of the RAS genes has been observed in a variety of malignancies and has been reported at high frequency in a number of leukemias. RAS mutations occur at an approximate frequency of 25% in acute myeloid leukemia, 30% in myeloma and 6-20% in acute lymphoblastic leukemia. The highest incidence reported, however, is from studies on the myelodysplastic syndromes, with frequencies of approximately 30-40% (Table 2). Most studies agree that mutations in the N-RAS gene predominate over K-RAS and H-RAS mutations and that in the larger studies, RAS mutations are preferentially found in either CMML or in groups demonstrating monocytosis. This mirrors the high frequency of RAS mutations associated with FAB type M4 of the AMLs. The role of the mutated RAS genes in the pathogenesis of MDS and in leukemogenesis remains controversial. The association of RAS mutation with the preleukemic state in MDS infers a role for these lesions in the leukemic process. A positive correlation between the presence of RAS mutations and acute leukemic transformation has been reported, including a more recent and larger study carried out by Paquette and co-workers which showed this correlation to hold even after stratification on percentage blasts in the bone marrow. Conflicting findings have been reported where the presence of RAS mutations offered no prognostic information. Indeed, RAS mutations have been shown to be lost during the progression from MDS to acute leukemia or upon the relapse of AML with a more aggressive clone. Whether RAS gene mutations represent an early or late event in leukemogenesis remains unclear. In one case report, a patient in remission from Hodgkin’s disease but who was hematologically normal, although at risk of developing secondary therapy related leukemia, harbored a RAS mutation. This patient also had a 7q deletion and abnormal progenitor growth in the absence of clinical symptoms. In addition, other patients at risk of developing secondary therapy related leukemia have been found to have RAS mutations. Taken together, these data suggests that, at least in some cases, RAS mutations represent an early lesion in the leukemic process, although others report the appearance of RAS mutations late in the course of the disease. Within individual MDS patients, RAS mutations have been reported in a multipotent stem cell where the mutation is associated with all cell lineages; however, in some other patients a RAS mutation is present in

<table>
<thead>
<tr>
<th>Author (year)</th>
<th>RAS+/Total</th>
<th>RAS+/RAS-</th>
<th>Mutation</th>
</tr>
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<tbody>
<tr>
<td>Lyons, 1988</td>
<td>3/34</td>
<td>0/4</td>
<td>K-RAS 12</td>
</tr>
<tr>
<td>Padua, 1988</td>
<td>20/50</td>
<td>8/4</td>
<td>H-, K-, N-RAS 12, 13, 61</td>
</tr>
<tr>
<td>Hirai, 1988</td>
<td>2/2</td>
<td>2/0</td>
<td>N-RAS 13</td>
</tr>
<tr>
<td>Yunis, 1989</td>
<td>11/27</td>
<td>8/3</td>
<td>N-RAS 12, 13</td>
</tr>
<tr>
<td>Lubbert, 1992</td>
<td>3/9</td>
<td>3/1</td>
<td>N-RAS 12</td>
</tr>
<tr>
<td>Paquette, 1993</td>
<td>20/220</td>
<td>8/4</td>
<td>N-RAS 12, 61</td>
</tr>
<tr>
<td>Neubaur, 1994</td>
<td>3/27</td>
<td>1/10</td>
<td>N-RAS 12, 61</td>
</tr>
<tr>
<td>Honike, 1994</td>
<td>6/35</td>
<td>4/8</td>
<td>N-RAS 12, 13</td>
</tr>
</tbody>
</table>

*The number of analyzed patients with RAS gene mutations/the total number of patients analyzed. °Transformation to acute leukemia in patients followed who harbored RAS mutation without RAS mutations. The specific RAS gene mutated and the codon(s) demonstrating mutations.
only myeloid lineages and not in lymphocytes. In these cases the clonal relationship of all three lineages was demonstrated by X-chromosome inactivation analysis, indicating that the mutation was acquired later in the course of the disease. The specific relationship of the RAS gene mutations with stage of disease and hence disease progression remains inconclusive. In a study by Hirai et al., an increasing signal with mutant N-RAS specific probes was observed in two patients upon disease progression, both of whom harbored a deletion of chromosome 5 at presentation. The investigators suggested that this chromosome abnormality may provide an early stage proliferative advantage, whereas the acquisition of a RAS mutation represented a secondary event leading to the progression of disease and ultimate leukemic transformation. The link between RAS mutations and abnormal karyotypes in MDS has been noted by other investigators: an increased incidence of mutant RAS in those patients demonstrating abnormal karyotypes has been reported and in another report, a positive correlation of the incidence of RAS mutations with leukemic transformation was revealed only when these data were combined with chromosomal abnormality details. In a more recent study, the co-existence of RAS activation by point mutation and monosomy 7 in MDS patients, but not in de novo AML patients, suggests that these lesions may co-operate in the multi-step process of leukemogenesis. These data could suggest that acquisition of a RAS mutation alone is not sufficient to induce clinical pre-leukemia but rather requires accumulation of other molecular aberrations. The exhibition of RAS mutations in two hematologically normal individuals and in hematologically normal individuals at risk of developing secondary therapy-related leukemia supports this view. The simultaneous activation of different RAS genes or of RAS with the tumor suppressor gene, p53, have been described and this further suggests that activation of single RAS genes is not always sufficient for complete pre-neoplastic or neoplastic transformation in vivo. It must be added, however, that simultaneous activation of both RAS and p53 genes is a rare event. Although several studies have been dedicated to unravelling the role of RAS mutations in the pathogenesis and progression of MDS, few have addressed the question of whether p21RAS has a role to play in these conditions or indeed whether differential expression of p21RAS occurs in normal hematopoiesis. In one study by Kalmantis, a strong correlation was presented between the immunohistochemical staining of p21RAS in bone marrow smears and the more aggressive FAB groups of MDS. We have shown that over-expression of p21RAS occurs at a high frequency in MDS. This finding was associated with the low risk FAB types (RA and RARS), indicating that over-expression of p21RAS may represent an early event in leukemogenesis. It is clear that activation of RAS genes, or of their products, has a fundamental role to play in hematological malignancy, in MDS in particular. The prognostic significance of RAS mutations alone or in combination with other factors remains to be resolved. A 7-year follow-up of our MDS patients who were investigated for RAS, FMS and p53 mutations (Guinn et al., submitted) revealed that the presence of oncogene mutation had a significantly poor prognosis in terms of survival, and a multivariate analysis showed that patients with mutations had a poor outcome (Guinn et al., submitted).

The frequent occurrence of chromosome 5 deletions in MDS has focused attention on the genes mapped to chromosome 5. The macrophage colony-stimulating factor receptor gene, c-FMS/CSF-1 receptor is localized to 5q31-33 and is a member of the family of growth factor receptors exhibiting ligand-induced tyrosine kinase activity. The FMS gene is the cellular homolog of the Susan Mc Donagh feline sarcoma virus, which exhibits constitutive tyrosine kinase activity in the absence of ligand and can induce a transformed phenotype in hematopoietic cell lines. Two mutations of the FMS gene have been identified at codons 969 and 301 in both MDS and AML at frequencies of 13-20% and 10-25%, respectively. Mutation at codon 301 produces a receptor with tyrosine kinase activity and transforming ability, while alterations at codon 969 enhance transformation in the presence of CSF-1 ligand. Despite this, the most common mutation found in these studies, as with our other studies investigating FMS mutations in patients at risk of secondary leukemia, was at codon 969 with gene product replacement of tyrosine with cysteine. In support of this finding, in vitro experiments, using retrovirally transfected FMS genes harboring mutations, have demonstrated transformation of the factor-dependent cell line FDC-P1 rendering it factor-(IL-3) independent. Mutation of p53 at codon 969 was not necessarily lead to morphologically detectable changes in hematopoiesis in vivo, but it can be speculated that this individual may be more susceptible to malignant transformation. Similarly, hemizygous deletion of c-FMS sequences, observed in some MDS patients with 5q- syndrome, does not necessarily indicate a critical role in the pathogenesis of 5q- syndrome; however, homozygous deletion was detected in 4 of 10 MDS patients in another
Recent advances in the molecular study of myelodysplastic syndromes

Recent studies have revealed the frequency and modes of activation of various genes encoding cytokine and tyrosine kinase receptors, all of which may contribute to the pathogenesis of MDS. The EVI-1 gene encodes a DNA-binding protein not normally expressed in blood or bone marrow cells, but in virus-induced murine hematological leukemias and human leukemias with translocations involving chromosome 3q26. Transcriptional activation of the EVI-1 gene is normally associated with 3q26 abnormalities and abnormal differentiation in AML: frequent expression of EVI-1 has been demonstrated in patients with post-MDS AML and MDS with tri-lineage dysplasia regardless of 3q26 aberrations. This suggests that the role of EVI-1 activation may differ in AML and MDS or post-MDS AML. A high incidence of EVI-1 expression was shown to occur in the bone marrow blasts of RAEB and RAEB-t MDS patients, although a significant correlation with other clinical factors was not observed, suggesting that abnormal EVI-1 expression is not a major determinant of ineffective hematopoiesis in MDS.

Since abnormal expression of the EVI-1 gene can occur regardless of 3q26 abnormalities, expression studies of this gene do not provide for detection of 3q26 chromosomal abnormalities. A translocation t(8;21)(q22;q22), found occasionally in MDS and more frequently in AML, results in the fusion of the AML1 gene (22q22) to the ETO gene (8q22). The detection of a chimeric AML1/ETO transcript by reverse transcription-PCR offers a fast and accurate diagnosis of t(8;21). The molecular consequences of a t(5;12) is a fusion pattern of AXL is detectable in normal bone marrow blasts of RAEB and RAEB-t MDS patients, although a significant correlation with other clinical factors was not observed, suggesting that abnormal EVI-1 expression is not a major determinant of ineffective hematopoiesis in MDS.

The precise role of AXL in myeloid malignancy remains to be determined.

The human TEC gene has recently been mapped to chromosome 4p12. High expression of its gene product, a non-receptor type protein tyrosine kinase, has been observed in MDS patients, with highest expression in the bone marrow progenitor cells. Other non-receptor type tyrosine kinases reported to be highly expressed in hematopoietic malignancy include the hematopoietic cell specific kinase, HCK. The aberrant expression of these genes may contribute to the ineffective hematopoiesis observed in MDS. Although excessive protein tyrosine kinase activity can result in malignant transformation, tyrosine phosphatase activity is also an important regulator of cell growth and differentiation. Amplification of a hematopoietic
specific tyrosine phosphatase (HePTP) has been described in MDS characterized by myeloid hypoplasia and monocytosis. Elevated protein expression was also observed in the diseased myelomonocytic cells and gene transfer of HePTP into NIH3T3 cells causes perturbation of cell morphology and growth. It is therefore likely that amplification and/or over-expression of this gene may play a key role in the disruption of normal cell growth in myelodysplasia.

The cytokine receptor superfamily includes genes encoding receptors for IL-3, IL-4, IL-5, IL-6, IL-7, IL-9, GM-CSF, G-CSF, erythropoietin, LIF, oncostatin M, growth hormone and prolactin. The c-MPL gene encodes a new member of this family of cytokine receptors, the thrombopoietin receptor. Thrombopoietin stimulates megakaryocytic progenitor expansion and differentiation. Expression of c-MPL was found to be increased in 26 of 51 AML patients of interest in the mechanism of the involvement of this chromosome 5q in MDS has aroused considerable demonstration homozygous or hemizygous loss of one or more of these genes may contribute to the pathogenesis of MDS, e.g. some patients with MDS have demonstrated homozgyous or hemizygous loss of the FMS (CSF-1 receptor) gene. Other chromosome abnormalities that have been implicated in MDS involve chromosome 11: inv(11)(p13q23), del(11q), t(2;11) and t(11;21). In the study by Mitani the c-H-RAS gene locus, located at chromosome 11p15, was shown to be transferred to the long arm of the rearranged chromosome 11 in one MDS patient, whilst the H-RAS gene was deleted in the rearranged chromosome in another MDS patient. The c-ETS-1 gene, mapped to 11q23, was demonstrated to have translocated to the short arm in the rearranged chromosome 11 in both of these patients. Studies of chromosome 11 in the pathogenesis of leukemia have also included the investigation of chromosomal DNA methylation status. Due to its implication in the regulation of eukaryotic gene expression, DNA methylation is thought to play a role in tumorigenesis and establishment of phenotypic characteristics of tumor cells. Hypermethylation of the calcitonin gene on the short arm of chromosome 11, used as a marker for DNA methylation status, has been demonstrated in most acute leukemias and blast crisis chronic myeloid leukemia (CML). In MDS, one study has shown that hypermethylation of the calcitonin gene is an early event in the pathogenesis of the disease.

In a study by Srivastava et al. an MDS patient harboring the translocation t(5;12)(q31;p12) demonstrated over-expression of the K-RAS2 gene, which maps at 12p12. The frequent occurrence of RAS gene mutations in MDS has been well docu-
ment; however, the evidence discussed here suggests that the effect of abnormal RAS genes may be additionally exerted by chromosomal aberration. Deletions of the long arm of chromosome 20 have been identified in approximately 5% of patients with MDS.\cite{106} Fluorescence in situ hybridization studies have identified a region from 20q11.2 to q12 that was deleted in 18 of 19 patients with 20q deletions.\cite{107} Genes which have been mapped to this region include hematopoietic cell kinase,\cite{108} phospholipase C,\cite{109} topoisomerase 1,\cite{109} and phosphotyrosyl phosphatase 1B,\cite{110} all of which have roles in the regulation of cell growth.

Trisomy 8 abnormality has been found in MDS patients at a frequency of up to 20%.\cite{106,111} Recently, the detection of trisomy 8 by FISH in bone marrow smears, prior to the demonstration of marked myelodysplastic changes, has suggested that this analysis should prove to be a useful aid to diagnosis of MDS.\cite{112} Genes mapped to chromosome 8 include MYC and MOS and more recently LIN, a member of the c-SRC gene family,\cite{113} and the tumor virus integration site, PVT-1.\cite{114,115} A role for these genes in the pathogenesis of MDS has not been reported to date. The loss of chromosome 7 (monosomy 7 and del7q) is a recurring aberration in MDS and AML where the monosomy 7 syndrome is particularly associated with childhood leukemia.\cite{116} The deletions in chromosome 7 extend from q11 to q36, with the critical region extending from 7q32 to q34.\cite{117} Genes known to be deleted in this syndrome include the MET protooncogene, mapped to 7q31.\cite{118} Other genes localized to chromosome 7q include the multidrug resistance protein, p-glycoprotein, and the erythropoietin gene.\cite{119} EVI-1 expression has been frequently associated with chromosome 7 deletion and 3q26 abnormalities.\cite{71,120} Expression of the EVI-1 gene has been demonstrated in patients with MDS without 3q26 anomalies, especially those diagnosed with RAEB and RAEB-t but not in those with CMML.\cite{121} This suggests a role for EVI-1 gene expression in the pathogenesis of MDS in association with chromosomal structural abnormalities other than those involving 3q23. The ever-increasing list of genes shown to map to various regions on abnormally structured chromosomes has broadened the scope for molecular investigation into the pathogenesis of MDS.

**Colony stimulating factor therapy in myelodysplastic syndromes**

In view of the average advanced age of MDS patients and the poor response to therapy, no standard therapy for the majority of patients exists other than supportive care. For younger patients (under 50 years) the preferred treatment is allogeneic bone marrow transplant from a HLA-identical sibling or a closely/fully matched unrelated donor.\cite{122} Combined chemotherapy using drugs such as cytosine arabinoside (Ara-c), daunorubicin, thioguanine and/or vincristine has been used to in an attempt to induce complete remission (CR), and although the response rate is reported as 15-64%, the rate of CR was lower in MDS and secondary AML than in de novo AML patients.\cite{123} This higher resistance to these drugs in MDS may be due to the higher level of drug resistance-associated protein, p-glycoprotein, the product of the MDR-1 gene.\cite{124} This high level of p-glycoprotein was associated with immature progenitor cells from advanced MDS patients (RAEB and RAEB-t).\cite{125} Since MDS is characterized by inappropriate maturation of the progenitor cells, treatment with differentiation agents such as low dose Ara-c,\cite{126} cis-retinoic acid,\cite{127,128} or vitamin D3\cite{129} has been employed. The effects of these treatment regimens have been found to be either short-lived with toxicity, as in the case of low dose Ara-c,\cite{126,128} or inadequate in the induction of any significant improvements, as with the other differentiation agents.\cite{128} In older patients an attempt has been made to modify dominant cytopenias by using human growth factors such as GM-CSF, G-CSF, interleukin (IL)-3, IL-6 and erythropoietin (epo). Since members of the family of colony stimulating factors have the ability to induce the proliferation and differentiation of normal and leukemic progenitor cells in vitro, the efficacy of these agents in the treatment of MDS has been evaluated by phase I and II clinical trials. A good improvement of neutrophil counts in the majority of MDS patients was demonstrated upon GM-CSF and G-CSF treatment.\cite{122,126} In the case of GM-CSF therapy, although there was a good initial response for the relatively low risk MDS patients, only 35% of the patient group completed the treatment. Therefore it is difficult to assess clinical outcome in terms of transformation to AML or survival. IL-3, which stimulates the proliferation/differentiation of pluripotent hematopoietic cells, showed increased bone marrow cellularity with stimulation of all three lineages.\cite{127} Other studies have shown, however, that although improvements in neutrophil counts were observed upon IL-3 therapy, there were only limited responses in the other lineages.\cite{129,130} Multilineage effects of IL-3 treatment were observed in only 25% of patients and it was suggested that the optimal use of IL-3 cytokine therapy would be in combination with other agents.\cite{140} The co-stimulation of growth factors, including GM-CSF with IL-3,\cite{141} has demonstrated an enhancement of GM-CSF activity in vitro colony formation assays on MDS bone marrow cells. No improvement in survival was observed in MDS patients using GM-CSF combined with low dose Ara-c compared with Ara-c treatment alone. The most adverse side effects (including hemorrhage and infection) were, however, due to the low dose of Ara-c. A phase III trial
using G-CSF to treat high risk MDS patients was performed by Greenberg et al. \(^{142}\) The incidence of progression to AML was not altered in the treated versus the control patients; however, an improved neutrophil count was observed for most patients in the study. Considering that dyserythropoietic anemia is the major clinical problem in the low-risk MDS group, the results of studies using epo therapy have been somewhat disappointing with response rates varying in the range of 2-50% with an average value of about, 15%. \(^{143}\) This is not surprising, however, since in vitro studies have shown less than optimal growth of BFU-E in response to epo treatment, even under closely controlled conditions in culture. \(^{144,145}\) Synergy of effects has been documented for epo and G-CSF in vivo with a response rate of 45%. \(^{146,147}\) In these studies the response was better for those patients with low endogenous epo levels, as was suggested by others. \(^{148}\) In a study by Runde \(^{149}\) the response rate to epo therapy was 10-25%, which was not enhanced by a combination of epo and GM-CSF. Further studies are required in order to determine any additional benefits of combining epo therapy with other cytokines.

Studies have indicated a role for mast cell growth factor, the c-KIT gene ligand, in improving the colony forming capacity of myelodysplastic progenitor cells. \(^{150,151}\) A synergistic effect of MGF and epo has also been demonstrated using in vitro studies where a diminished requirement for epo in erythroid differentiation was observed. \(^{152}\) Although inappropriate development is the key feature of myelodysplastic progenitor cells, autonomous growth, independent of growth factor stimulus, has been described in myeloid leukemias. \(^{153,154}\) Autocrine or paracrine production of growth factors such as MGF or the deregulation of growth factor receptors, for example encoded by c-KIT, may be responsible. Elevated c-KIT expression both at RNA and protein levels has been shown in bone marrow CD34-positive cells from MDS patients. \(^{155}\) The potential of this growth factor in the treatment of MDS remains to be established. No difference in the levels of GM-CSF or IL-3 receptors was observed in MDS patient bone marrow when compared with normals, \(^{156}\) although increased levels of circulating GM-CSF, but not IL-3, were measured in approximately 25% of patients in this study. This suggests that a functional alteration of the growth factor receptors rather than expression levels may also be important. This idea was supported by work carried out by Dong et al., \(^{157}\) where point mutations in the G-CSF receptor gene were identified in two AML patients with a history of severe neutropenia. In one patient the mutation was also found in the neutropenic stage, prior to progression to AML. In this study, the mutant receptor interfered with terminal maturation of cells treated with G-CSF.

In conclusion, stimulation of cytokine receptors with combinations of growth factors may prove to be a worthwhile therapeutic strategy for the treatment of MDS, but only in patients without receptor defects. Evaluation of these strategies needs to be conducted through further coordinated and comparative studies.

**Molecular analysis of clonality in myelodysplastic syndromes**

The presence of pancytopenia in MDS suggests that, in these cases, all cell lineages are affected by the myelodysplastic lesion(s). Characterization of an MDS clone exploits the high frequency of activated oncogenes and chromosomal aberrations. \(^{\text{RAS}}\) mutational activation has been reported both in early and late stages of the disease in up to 50% of cases. The finding of \(^{\text{RAS}}\) mutations in the cells of all lineages of the peripheral blood would suggest monoclonality. This was demonstrated by Janssen et al. who detected K- and N-RAS gene mutations in two cases of CMML. \(^{158}\) Since all lineages were affected by these mutations it could be concluded that myelodysplasia in these patients was initiated in the aberrant stem cell and constituted a clonal disease. Numerical chromosomal aberrations, commonly trisomy 8, monosomy 7 or deletions in the long arm of chromosome 5 (5q-) can be used to characterize the MDS clone. \(^{159}\) The disadvantage of applying these methods to MDS clonality investigations is that these lesions may arise at various stages in the disease. This problem has been overcome by the use of the X-chromosome-linked restriction fragment length polymorphism assay (RFLP), which is based on differences in X-chromosome inactivation, a DNA methylation dependent process. The polymorphic hypoxanthine phosphoribosyltransferase (HPRT) and phosphoglycerate kinase (PGK) genes or a locus recognized by the M27\(\beta\) probe are commonly used to study clonality in small populations of cells from the various bone marrow and peripheral blood cell lineages. A monoclonal pattern in MDS has been reported by several investigators, \(^{159,160}\) whilst polyclonal T-lymphocytes in MDS have also been reported. \(^{161}\) In addition, polyclonal granulocytes and lymphocytes have been observed in MDS patients with monoclonal erythrocytes. \(^{162}\) In a study by Asano \(^{163}\) X-chromosome inactivation analysis of in vitro cultured progenitor cells showed the existence of non-clonal progenitor cells which produced erythroid and non-erythroid colonies upon growth factor stimulation.

In conclusion, although the studies using activation of the \(^{\text{RAS}}\) oncogene indicated that MDS is a clonal disease, the investigations using RFLP analysis provide evidence that monoclonality is not a feature of all cases of MDS.
Premature cell death in myelodysplastic syndromes: molecular control

Apoptosis or programmed cell death, first described by Kerr, Wyllie and Currie in 1972, is an energy dependent process of cell suicide characterized morphologically by chromatin condensation, nuclear disintegration and cell shrinkage followed by the formation of membrane bound apoptotic bodies. Apoptotic cells do not induce an inflammatory response but are targets of immediate phagocytosis by adjacent macrophages, surrounding epithelial cells or in solid tumors, by neighboring tumor cells. Apoptosis plays a role in a number of physiological processes including embryonic development, tissue maintenance and clonal selection in the immune system. Recent reports have revealed that persistent peripheral blood cytopenias in combination with the presence of a normo- or hyper-cellular marrow in MDS can be accounted for by premature intramedullary cell death by apoptosis. A histological study of MDS bone marrow biopsies by Clark and Lampert revealed a significant amount of apoptosis, the index of which was greater than that shown in normal biopsies. A study of cell kinetics and apoptosis in MDS bone marrow cells revealed a high rate of cellular proliferation which coincided with an incredibly high incidence of apoptosis in three lineages. Large numbers of S-phase cells were undergoing apoptosis as well as stromal cells in the bone marrow microenvironment. It was concluded from this study that extensive apoptosis in the MDS cells effectively cancels out the high birth rate, resulting in ineffective hematopoiesis.

A number of gene products have been implicated in the control of or the participation in apoptosis. p53, c-MYC and BCL-2 have been shown to play central roles in the regulation of programmed cell death and in cellular proliferation. Wild type p53 can induce apoptosis in myeloid cell lines and DNA damaging agents such as ionizing radiation require p53 activity to induce apoptosis. WAF1/CIP-1, an inhibitor of cyclin dependent kinases and target of p53 and several other genes including BAX and MDM-2, has been implicated in the regulation of programmed cell death. Continued expression of c-MYC in the presence of growth arrest signals leads to the acceleration of apoptosis. Since c-MYC has normally been associated with cellular proliferation it became clear that this gene can induce both signals, proliferation and death, depending on other cellular signals, e.g. the presence or absence of growth factor stimuli. The BCL-2 gene is a specific suppressor of apoptosis in a range of cell types, the product of which acts in two ways: to suppress the activity of oxygen free radicals that induce lesions in DNA, proteins and lipids, and to inhibit sustained increases in cytosolic and nuclear calcium levels, implicated in the activation of calcium-dependent endonucleases. Several BCL-2 related genes have now been identified, some of which act in a similar way in the suppression of apoptosis, i.e. BCL-xl, and others which induce apoptosis, namely BCL-xs, BAX and BAK. BAX, a dominant negative inhibitor of BCL-2, forms homodimers and heterodimers with BCL-2. Given that BAX elicits an opposing effect to that of BCL-2, the ratio of BCL-2 to BAX could be important in determining cellular response to apoptotic stimuli. The role of BCL-2 in the regulation of apoptosis is evidenced by its interaction with other genes involved in cell proliferation and differentiation. Activation of the RAS signalling pathway (shown to inhibit apoptosis in KS62 cells and in rat fibroblasts) caused upregulation of BCL-2 and BCL-xl, but did not affect BAX expression. This gives a clue as to the mechanism of cell survival induced by growth factors GM-CSF and IL-3, which act via activation of the RAS signalling pathway. Functional synergy in the suppression of apoptosis has been reported for the serine/threonine kinase, RAF-1 and BCL-2. Given the established relation of R-RAS with BCL-2 and BCL-2 with RAF-1, R-RAS may be a facilitator of the BCL-2/RAF interaction.

Despite the rapid progression into the understanding of molecular events underlying cell death by apoptosis, limited studies have examined the role of these key genes in MDS. The tumor suppressor gene, p53, maps to chromosome 17p and is often inactivated through deletion and/or mutations in solid tumors, and to a lesser extent in hematopoietic malignancies. Chromosome 17p deletion is not uncommon in MDS and is associated with more mature granulocytes, although patients with 17p abnormalities do not always have p53 mutations. In MDS, mutational inactivation of p53 is rare with an incidence of less than 5% in most cases but found in up to 15% of cases with a more progressed phenotype. In a recent study, however, the presence of p53 mutations in MDS served as a significant prognostic indicator of response to chemotherapy and survival. No positive correlation has been observed between the incidence of p53 and RAS mutations in MDS, indicating a heterogeneity in oncogenic mechanisms involved in MDS. Lepelley reported the positive expression of BCL-2 in MDS blasts but more differentiated cells were always negative. In normal myeloid progenitors, BCL-2 protein expression was inversely related to maturation and was high in CD34+ cells. In the study by Lepelley et al., however, no correlation was observed between BCL-2 expression and FAB subtype of MDS, CD34 expression and p-glycoprotein expression. A strong correlation between weak BCL-2 expression and the presence of a p53 mutation was observed, indicating the possibility of down-regulation of BCL-2 expression by p53 mutation. Further studies on...
the possible role of the deregulation of BCL-2 and related genes in inducing apoptosis in myelodysplastic bone marrow are necessary. Interleukin-1 beta converting enzyme (ICE) is implicated in apoptosis based on its structural similarity to the programmed cell death gene, CED 3, in Caenorhabditis elegans195 and the induction of apoptosis in fibroblasts transfected with recombinant ICE.196 A role for this protease in the apoptosis of myelodysplastic cells has not yet been addressed. The role of programmed cell death in normal hematopoiesis and in myelodysplastic marrows needs to be addressed fully in order to increase our understanding of how the function of genes such as p53, c-MYC and BCL-2 contribute to the pathogenesis of MDS. The P39 cell line, derived from a MDS patient in leukemic phase, has been shown to undergo marked apoptosis in vitro in the presence of the differentiation inducer all-trans retinoic acid.197 This in vitro system should serve as a suitable model for the study of apoptosis in relation to impaired differentiation in MDS with a view to studying the role of the apoptosis-related genes (Figure 1).

**Conclusions**

A number of candidate genes of interest in the pathogenesis of MDS have been described. It appears that no single event is solely responsible for the clinical development of these syndromes but that they occur rather by an accumulation of chromosomal anomalies and individual gene defects. In some cases, the defective genes have been identified, but so far there is little information as to what role the deregulated genes or gene products may have in the pathogenesis of MDS. Some work on the effect of mutant RAS expression on the growth and differentiation of primary hematopoietic cells has been carried out in order to address its possible role as an initiator of the disease. Mutant RAS has been shown to potentiate myeloid growth in long-term bone marrow culture198 and to block erythroid differentiation of human cord blood cells.199 The latter result is of particular interest given that erythroid dysplasia is one of the most common features of MDS. The diversity of abnormalities already identified in myelodysplasia, together with the work that still needs to be carried out in order to define their respective roles in the disease process, seems an awesome task. However, many of these abnormalities may cause disease through common mechanisms. For instance, RAS signalling may be affected by a number of disparate mechanisms in addition to mutation and overexpression; loss of RAS GAP activity is an obvious example.57 In addition, RAS also transduces signals from many hematopoietic growth factor receptors.200,201 Therefore alteration in the activity of these receptors, either through mutation59 or overexpression,63 will also lead to aberrant RAS activation. Other com-
common pathways influenced by the accumulation of individual gene defects in MDS will become clear as we understand the interactions of the genes discussed in this review. The challenge that follows is to develop therapeutic strategies that can restore normal function to these signalling pathways or to specifically destroy defective cells. Amongst the proposed approaches has been the blockade or reversal of oncogenic p21RAS mutants by specific inhibitors of downstream pathways of RAS using NF-1 fragments or N-terminal fragments of the RAF-1 kinase, which lies immediately downstream of p21RAS. The potential of these agents in conditions such as MDS remains to be revealed.

References


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