Myelodysplastic Syndromes

NRAS, *FLT3* and *TP53* mutations in patients with myelodysplastic syndrome and a del(5q)

Mutations of the *NRAS* and *TP53* genes and internal tandem duplication (ITD) of the *FLT3* gene are among the most frequently observed molecular abnormalities in the myelodysplastic syndromes (MDS) and acute myeloid leukemia (AML). We sought to determine the incidence of these abnormalities in patients with MDS and a 5q deletion. *NRAS* and *FLT3* mutations are uncommon in MDS patients with a 5q deletion and *TP53* mutation is associated with the more advanced MDS subtypes.

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Myelodysplastic syndromes are a heterogeneous group of acquired clonal disorders of hematopoiesis. Clonal karyotypic abnormalities occur in approximately 50% of primary MDS cases; the del(5q) is the most frequently reported abnormality and is observed in 10-15% of patients.[1] The del(5q) occurs as the sole karyotypic abnormality in the 5q- syndrome.

RAS, *FLT3* and *TP53* genes play important roles in the regulatory processes that govern proliferation, differentiation and apoptosis. Abnormalities in these three genes have been implicated in the pathogenesis of MDS and AML. The most common molecular abnormality in MDS is activation of the *RAS* proto-oncogenes, reported in 4-48% of patients.[2,3] *TP53* point mutations are detected in 5-10% of MDS patients[3,4] and *FLT3* ITD mutations have been reported in approximately 3% of MDS patients.[5] We sought to determine the incidence of *NRAS*, *FLT3* and *TP53* mutations in patients with MDS and a 5q deletion and to investigate whether the frequency of these mutations differed between patients with the 5q- syndrome and patients with a del(5q) and more advanced stages of MDS.

Peripheral blood samples were obtained from 40 patients with MDS; 24 with refractory anemia (RA) (including 20 with 5q- syndrome), 1 with RA with ringed sideroblasts (RARS), 11 with RA and excess blasts (RAEB), 2 with RAEB in transformation (RAEBt), 1 with chronic myelomonocytic leukemia (CMML) and 1 AML with a preceding MDS. All patients had a del(5q); 25 patients had del(5q) as the sole karyotypic abnormality, 9 patients had del(5q) plus 1 or 2 other karyotypic abnormalities and 6 patients had a complex karyotype. Sequences spanning codons 12 and 13 and codon 61 of *NRAS*, exons 5-8 of *TP53* and exon 14, intron 14 and exon 15 of *FLT3* were amplified by polymerase chain reaction (PCR). The sequences of all primers have already been published.[6] PCR products were purified and directly sequenced using the BigDye Terminator version 3 kit (Applied Biosystems). The sequencing methodology detects all significant mutations, although mutations in minor clones (less than 15%) may not be detected.

ITD of *FLT3* was not observed in any of the 40 MDS patients. This finding is consistent with the published low frequency.[5] Five different *TP53* mutations were observed in 3/40 (7.5%) patients (Table 1). The reported incidence of *TP53* mutations in MDS is 5-10%; again our data are consistent with these reports. Studies have shown that *TP53* mutations occur predominantly in the poor-risk FAB subtypes of RAEB, RAEBt and CMML; the three patients with mutations in this study were all classified as having RAEB. Christiansen *et al.* have shown that *TP53* mutations are significantly associated with deletion or loss of 5q in therapy-related-MDS and t-AML after previous treatment with alkylating agents and are associated with a poor prognosis.[7] Interestingly, Castro *et al.* suggested that the loss of chromosome 5q13.3 sequences confers a proliferative advantage to a dysplastic clone that can be fully transformed upon acquiring a mutation of the *TP53* gene.[8]

The most interesting observation in the present study was the absence of *NRAS* mutations. Various studies have reported very different *RAS* mutation rates, from 4% to 48%.[2,3] To confirm that we could detect such mutations by direct sequencing, three DNA samples known to carry *NRAS* mutations, cell line MDS-92 and two samples from patients, were sequenced. An *NRAS* mutation was clearly detected in each sample. The apparent discrepancy between our absence of *NRAS* mutations and some high reported frequencies may rest in the selection of patients, or possibly that the earlier studies using dot blot and oligonucleotide hybridization methodology overestimated the incidence of this mutation.[9] The striking absence of any *RAS* mutations in this study is strong evidence that the mutation plays no role in either initiation or transformation of these leukemias. We speculate that other biochemical pathways, rather than an aberrant *RAS* pathway, may play a role in leukemogenesis in MDS patients with the del(5q).

None of the 20 patients with 5q- syndrome had mutations of the three genes. A recent study investigated tumor suppressor gene deletion by fluorescence *in situ* hybridization in a similar group of leukemias, and again no abnormalities in the 5q- syndrome group were observed.[10] Taking these data together, it does seem that the stability of the 5q- syndrome may be due to the absence of other additional abnormalities. The transformation of MDS into AML in some patients may well be due to *TP53* mutations, but clearly there are still additional genetic abnormalities to discover.

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Table 1. MDS patients with mutations in the *TP53* gene.

Pt	*Sex/Age*	*FAB*	*Karyotype*	*Exon*	*Codon*	*Type*	*Nucleotide*	*Amino Acid*
KB	F/82	RAEB	45XX,-7,-22, del(5)(q13q33),t(6;12)(q13;p12),+mar	6	192	nonsense	C → T	Gln → stop
KB	F/82	RAEB	45XX,-7,-22, del(5)(q13q33),t(6;12)(q13;p12),+mar	6	220	missense	A → G	Tyr → Cys
DS	F/85	RAEB	45XX,del(5)(q13q33),-7, del(12)(p11p12)	7	238	missense	G → A	Cys → Tyr
DS	F/85	RAEB	45XX,del(5)(q13q33),-7, del(12)(p11p12)	7	248	missense	G → T	Arg → Leu
BL	M/58	RAEB	43-45,XY,del(5)(q31), der(7)t(7;12)(q22;q1?3), -12,-13,-19,?del(20)(q1?3)	8	273	missense	G → A	Arg → His

RAEB, refractory anemia with excess of blasts. These mutations have all been previously reported in a variety of human cancers and are documented in the TP53 Mutation Database (http://p53.genome.ad.jp).

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Myelodysplastic Syndromes

Prognostic factors in myelodysplastic and myeloproliferative types of chronic myelomonocytic leukemia: a retrospective analysis of 83 patients from a single institution

We analyzed independent prognostic factors associated with survival and risk of evolution to acute leukemia in our series of 83 patients with previously untreated chronic myelomonocytic leukemia (CMML), with the aim of testing the validity of the stratification based on white blood cell (WBC) count, in myeloproliferative and myelodysplastic types of the revisited WHO classification.

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From 1989 to 2001, 643 adults were diagnosed with myelodysplastic syndromes (MDS) at our Institution; 83 (12.4%) of these had primary CMML. According to the limit of WBC count of 13×10^9/L, 46 had a myelodysplastic (MD) type and 37 a myeloproliferative (MP) type. The median follow-up of patients was 38 months. Bone marrow (BM) dysplasia was assessed according to previously established FAB criteria.[1] Cytogenetic studies were available for 48 patients. Different prognostic scores (Bournemouth, modified Bournemouth, Spanish, IPSS, Gonzalez-Medina score, Dusseldorf score, MDAPS)[2-4] were evaluated for risk distribution and relative impact on survival prediction. Clinical and hematologic features, tested for survival and rate of transformation to acute myeloid leukemia (AML), were compared by the χ^2 and Wilcoxon rank sum tests. Univariate analysis was estimated using Cox regression models. p values <0.05 were regarded as statistically significant in two tailed tests. SPSS software (version 10.00, SPSS, Chicago, USA) was used for statistical analyses. Significant independent variables were used to develop a multivariate model by the Cox method in order to identify independent prognostic relationships. The differences between the MD and MP groups were sex ratio, WBC, monocyte, platelet and lymphocyte count and hemoglobin level.

Trilineage dysplasia was evident in 35 MD (76%) and in 17 MP patients (46%) (p=0.005). A normal karyotype was observed in 38 patients (80%) while in 10 patients a +8 (4 MD and 1 MP patients), a del (13) (2 patients), a del (16) (2 patients), a -7 (1 patient) were identified.

Median survival was 20 and 17.4 months for MD and MP patients, respectively (p=0.007). The disease progression rate was higher for the MP type (29.7%) than for the MD type (15.2%, p=0.001). The median duration of the preacute leukemic phase was 16 and 14 months in the MP and MD variants, respectively (p=NS). At the time of our analysis, 12 patients with MD type (26%) and 19 with MP type (51%) had died (p=0.005). Death was related to AML progression (45%) or hemorrhagic (19%) and/or septic complications of BM failure (19%). Other causes of death were heart failure in 7 old patients receiving marked transfusional support (12%), liver failure (3%), and second neoplasia (2%). Survival after evolution into AML was 1.5 and 2 months for patients with MP and MD variants, respectively (p=NS). Twenty-one MP patients received hydroxyurea to control leukocytosis and 5 patients whose disease evolved into AML received intensive induction treatment. Transfusion requirements were higher in MP patients than in MD patients (59% vs 26%; p=0.003); the frequency of infections and hemorrhages was not significantly different in the two groups (p=NS). Only 6/14 patients with hemorrhages required platelet transfusions.

Sex, WBC > 13×10^9/L, lymphocytes >2.5×10^9/L, platelets < 100×10^9/L, and trilineage dysplasia were individually associated with shorter survival (Table 1). These parameters were entered in a multivariate analysis, but only trilineage dysplasia had independent prognostic value for survival (p=0.02).

BM blasts >5%, WBC count, neutrophils and lymphocytes >2.5×10^9/L, and presence of peripheral blood blasts, associated with AML progression in univariate analyses, were tested in a multivariate analysis but only the lymphocyte count had an independent prognostic value (p=0.01).

All scoring systems applied, except IPSS, stratified