Cytogenetics and interphase FISH for the diagnosis of patients with myelodysplastic syndromes (MDS)

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Myelodysplastic syndromes or myelodysplasia (MDS) are clonal hematological disorders characterized by ineffective hematopoiesis with no organomegaly. The French-American-British (FAB) classification of MDS into five groups: RA, RARS, RAEB, RAEB-T and CMML in 1979 provided useful but incomplete prognostic information (1). Most cooperative groups admit patients to clinical trials for AML with 20-30% blasts (RAEB-T). At the Fifth International Symposium on Myelodysplastic Syndrome held in Prague 21-24 April 1999, a proposal by W.H.O. morphology committee suggested a slight modification in MDS classification. The proposed classification will eliminate RAEB-T and will include two groups of RAEB: RAEB I for patients showing 1-10% blasts and RAEB II for patients with 11-20% blasts. The diagnosis of AML will include patients having >20% blasts.

Clonal chromosomal abnormalities can be detected in marrow cells from 40-70% of patients with primary MDS and in over 95% of patients with therapy related MDS (T-MDS) at the time of diagnosis (2). There appears to be a correlation between the frequency of chromosomal abnormalities with the severity of the disease. About 20-25% of patients with RA and RARS have clonal chromosomal rearrangements, whereas about 70% of patients with RAEB and RAEB-T show chromosomal rearrangements. In general, chromosomal rearrangements detected in MDS frequently include unbalanced translocations which often lead to the loss of genetic material. Hemizogosity for specific genes or chromosomal region is the hallmark of MDS even though some rearrangements like +8, -5/del(5q), -7/del(7q) and del(20q) are also seen in AML.

INTERNATIONAL PROGNOSTIC SCORING SYSTEM (IPSS)

In a recent study, the international MDS Risk Analysis Workshop combined cytogenetic, morphologic and clinical data from seven large risk-based studies, and defined the International Prognostic Scoring System (IPSS) for MDS (3).

Patients with "good outcome" or low risk had a normal karyotype, -Y alone, del(5q) alone, del(20q) alone or -4. Patients with an "intermediate outcome" had other single abnormalities or +8, and those with "poor outcome" or high risk had complex karyotype (>3 abnormalities) or chromosome 7q abnormalities. The median survival of patients within these three groups were 3.8, 2.4, and 0.8 years, respectively and the times for 25% of the patients to undergo evolution to AML were 5.6, 1.6, and 0.9 years. MDS patients with poor risk cytogenetics are at high risk of relapse after BMT. The most common abnormalities in pediatric MDS are monosomy 7, +8, and +21.

IPSS risk groups were recently validated with the study of 640 patients with MDS (4). Clonal chromosomal rearrangements were detected in 313 of the 640 studied patients (51%). Single chromosomal changes were found in 29% and complex karyotype in 14%. In addition to confirming IPSS risk groups this study suggested that abnormalities of 12p should be added to "good risk" and +8 should be removed from intermediate risk group to "poor outcome" group. The

rate of evolution to AML was high (more than 40%) for patients with involvement of 1q, monosomy 7, trisomy 11, involvement of 11q, 12q, trisomy 13, and trisomy 21. A low evolution (less than 20%) was found among the patients showing monosomy 5, involvement of 12p, involvement of 13q and loss of Y chromosome.

THE MOUNT SINAI SERIES

Between 1986 and May 1998 at the Mount Sinai Medical Center in New York we studied 862 patients with MDS. In our series 33.5% of patients had an abnormal karyotype. One of the reason for a slightly lower rate of the abnormal karyotype is that the Mount Sinai is a referral center for myelodysplasia, and at the time of our cytogenetic study, a number of patients were treated and were in either remission or the chromosome abnormality was suppressed. The frequency of clonal chromosomal rearrangements detected in our series is shown below:

CHROMOSOMAL ABNORMALITY	% of ABNORMAL	% of TOTAL
-7/del(7q)	29.4	10
-5/del(5q)	29	10
+8	21	7
+1q/1q rearrangements	18	6
+11/11q14-11q23 rearrangements	15.5	5
-20/del(20q)	15	5
-12/12p rearrangements	13	4.5
+21	12	4
-13/+13/del(13q)	10	3
17p rearrangements	5	2

Unlike other reported series, the fourth most frequent chromosomal abnormality in our series was rearrangements of chromosome 1 detected in 18% of 289 patients with abnormal karyotype (5). Trisomy 1 as the sole abnormality occurred in 3 patients, while translocations and deletions were identified in 50. Among these latter 50 patients, partial trisomy 1 was also detected in combination with either translocations or deletions in an additional 19%. Translocations (1;7) either in the form of der(1) or der(7) was most frequently observed followed by t(1;15), and t(1;17). Metaphase FISH analysis did reveal a dicentric chromosome in t(1;7). The most striking observation involved the identification of "jumping " translocation in 5 patients with MDS; 4 patients had a partial trisomy of chromosome 1, proximal to 1q12 band "jumping" to 11 different donor chromosomes and one patient had 7q "jumping" to chromosomes 1, 10, and 19. A "jumping" translocation was identified only once previously in a patient with CML in blast crisis among more than 8,000 patients with hematological disorders at our institution over the past 18 years.

We previously reported that trisomy 21 was most common but tetrasomy 21 either as i(21q) or in other translocations was observed in a number of patients' (6). In all MDS patients cytogenetically normal at diagnosis, detection of chromosome 21 abnormalities was associated with transformation/relapse. Immunophenotyping demonstrated that 89% of patients with MDS and chromosome 21 rearrangements had involvement of lymphoid lineage (50% had CD4/CD8, 22% had TdT and 17% had B-cell phenotype).

Patients with abnormalities of 17p were recently recognized to represent a specific cytogenetic-morphologic group, present in about 5% of MDS and AML showing dysgranulopoiesis with concomitant pseudo-Pelger-Huet nuclear hypolobulation and small vacuolated neutrophils. In most of these patients there is a loss of short arms of chromosome 17.

INTERPHASE FISH

Interphase fluorescence in situ hybridization (FISH) refers to detection of DNA sequences in non dividing, or interphase cells (7). The main advantages of interphase FISH over conventional metaphase cytogenetics are as follows: (1) no cell culture is required; (2) it is not dependent on a dividing cell populations or the cell cycle status and thus, it provides information at the constitutive level; (3) the results can be enumerated and quantified; (4) the procedure can be completed in 2-4 hours; (5) it is easily applicable to archival material; (6) together with immunological studies, the genotype and the phenotype can be viewed simultaneously; and (7) it provides information when conventional cytogenetics is uninformative.

In the last few years, interphase FISH has been used in conjunction with conventional cytogenetics as a diagnostic tool in MDS. Review of the recent literature revealed that at least 48 of 351 reported patients had discordant results between conventional cytogenetics and interphase FISH (8). Three major reasons for discordant studies were: (1) Interphase FISH detected 5% to 39% of cells with monosomy 7, an abnormality which was not observed by cytogenetics; (2) interphase FISH detected two hybridization signals of the same size in patients with –7, +marker; (3) substantially higher percentages of cells with +8 were observed by interphase FISH than by conventional cytogenetics, or occasionally, vice versa.

In order to determine whether patients with MDS and a normal karyotype have chromosomal abnormalities in non dividing cells, between 1997 and 1998

we performed interphase FISH, utilizing centromere enumeration probes for chromosomes 7 and 8 and locus specific probes for 5q31 and 20q12. Interphase FISH analysis of 17 patients with MDS and a normal karyotype did not reveal any cryptic rearrangements for these four tested loci. These observations suggest that once the chromosomal rearrangement occurs it has a proliferative advantage and is cytogenetically visible.

The use of FISH for identification of genetic events in single cells offers the possibility of correlating the genotype and cellular phenotype on a cell by cell basis. This is powerful method for detection of clonality, lineage involvement and events involved in pathogenesis of MDS. For example monosomy 7 was documented in myeloid cells characterized by CD11b and CD33 expression but not in T cells (CD3), B cells (CD20) and natural killer cells (CD 57) of seven patients with myelodysplasia (9). Combined morphological and FISH studies on an additional nine patients confirmed an earlier report (10). Another example is the use of interphase FISH to study development of myelodysplasia in children with Down syndrome (11). Trisomy 8 and monosomy 7 were documented by interphase FISH in normoblasts but not in myeloid or lymphoid cells . This observation suggests that MDS in these children may represent a unique disorder characterized by the proliferation of a progenitor cell capable of differentiating to megakaryocytic and erythroid lineages.

The new technique of multicolor FISH (M-FISH) in which simultaneous visualization of all human chromosomes, in different colors, is achieved (12), may provide in future an even more sensitive means of detecting cryptic chromosomal rearrangements.

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