Cancer Genetics 216-217 (2017) 120–127

Cancer Genetics

ORIGINAL ARTICLE

Prognostic classification of MDS is improved by the inclusion of FISH panel testing with conventional cytogenetics

Prajakta Kokate, Rupa Dalvi, Neeraja Koppaka, Swarna Mandava *

Cytogenetics division, SRL Diagnostic Ltd., Prime Square Building, Gaiwadi Industrial Estate, S.V.Road, Goregaon, Mumbai 400 062, India

Cytogenetics is a critical independent prognostic factor in myelodysplastic syndromes (MDS). Conventional cytogenetics (CC) and Fluorescence in situ hybridization (FISH) Panel Testing are extensively used for the prognostic stratification of MDS, although the FISH test is not yet a bona fide component of the International Prognostic Scoring System (IPSS). The present study compares the utility of CC and FISH to detect chromosomal anomalies and in prognostic categorization. GTG-Banding and FISH Panel Testing specifically for –5/–5q, –7/–7q, +8 and –20q was performed on whole blood or bone marrow samples from 136 patients with MDS. Chromosomal anomalies were found in 40 cases by CC, including three novel translocations. FISH identified at least one anomaly in 54/136 (39.7%) cases. More than one anomaly was found in 18/54 (33.3%) cases, therefore, overall FISH identified 75 anomalies of which 32 (42.6%) were undetected by CC. FISH provided additional information in cases with CC failure and in cases with a normal karyotype. Further, in ten cases with an abnormal karyotype, FISH could identify additional anomalies, increasing the number of abnormalities per patient. Although CC is the gold standard in the cytogenetic profiling of MDS, FISH has proven to be an asset in identifying additional abnormalities. The number of anomalies per patient can predict the prognosis in MDS and hence, FISH contributed towards prognostic re-categorization. The FISH Panel testing should be used as an adjunct to CC, irrespective of the adequacy of the number of metaphases in CC, as it improves the prognostic classification of MDS.

Keywords Fluorescence in situ hybridization, conventional cytogenetics, myelodysplastic syndrome

Introduction

Myelodysplastic syndromes (MDS) comprise a heterogeneous group of malignant disorders of hematopoietic stem cells. They are characterized by ineffective hematopoiesis, increased apoptosis, peripheral blood cytopenias, and propensity to evolve into Acute Myelogenous Leukemia (AML) (1).

MDS shows variable prognosis and therefore, the prognostic stratification of MDS patients becomes vital. The prognostication of MDS, presently relies on the International Prognostic Scoring System (IPSS), which risk stratifies patients based on three parameters, which are, number of cytopenias, bone marrow blast percentage and cytogenetic risk based on conventional cytogenetic analysis (2). Conventional cytogenetics has been proven to be an independent prognostic factor (3) and categorization into low- and high-risk MDS by cytogenetics serves as a basis for selecting the appropriate therapeutic option (4). Further, the number of anomalies present per patient i.e. whether an isolated, double or triple anomaly is present also predicts prognosis (3).

The heterogeneity of MDS is probably a reflection of the enormous variability of the cytogenetic abnormalities found in MDS. This genetic heterogeneity makes prognostic classification as well as delineation of the molecular background of cytogenetic aberrations in MDS challenging (4).

Considering the criticality of the cytogenetic profiling in MDS and the genetic heterogeneity observed in MDS, identifying

Received September 14, 2016; received in revised form January 6, 2017; accepted May 24, 2017.

* Corresponding author.
E-mail address: swarna@srl.in

2210-7762/$ - see front matter © 2017 Elsevier Inc. All rights reserved.
https://doi.org/10.1016/j.cancergen.2017.05.004

the most suitable algorithm to arrive at an accurate cytogenetic result has been a matter of open debate.

Conventional cytogenetics provides a complete picture of the genetic makeup and is considered the gold standard for correlating the genetic loci with the disorder and further understanding the molecular basis of the disease, delineating the genetic loci involved in the disorder, it is not, however, without drawbacks. Cytogenetic analysis can be hampered by low *in vitro* mitotic activity of cancer cells, poor chromosome morphology, considerable complexity (5). In such cases, Fluorescence in situ hybridization (FISH) analysis provides rapid and reliable detection of specific abnormalities directly implied in prognosis i.e. del 5q for lenalidomide therapy or unfavorable prognosis due to abnormalities of chromosome 7.

However, FISH probes are restricted to the detection of only specific abnormalities and genetic alterations beyond the scope of the FISH probes would therefore be completely missed.

The aim of our study was to compare the usefulness of fluorescence in situ hybridization and conventional cytogenetics to detect chromosome aberrations in MDS. FISH, although in its limited capacity can be a robust technique not only for identifying the most commonly seen anomalies, but also in recategorizing the patients into prognostic groups depending on the type of anomalies and the number of anomalies per patient.

Materials and methods

The study group included 136 patients (49 females and 87 males) with MDS referred to our Center during the period 2012–2015. The bone marrow/whole blood samples were subjected to Conventional Cytogenetics and Fluorescence in situ Hybridization. The study was approved by the Institutional Ethics Committee. Written consent was obtained from all the subjects.

Conventional cytogenetics

Cytogenetic studies were performed with unstimulated 0 h, 3 h, 24 h and 48 h cultures, using bone marrow aspirates or peripheral blood obtained from the 136 patients according to standard procedures. Chromosome preparations were G-banded using Trypsin and Giemsa and karyotypes were described in accordance with the International System for Human Cytogenetic Nomenclature 2013 (6).

Fluorescence in situ hybridization (FISH)

FISH studies were performed on fixed cells obtained from the cultures of conventional cytogenetics. The commercially available probes (Abbott/Vysis, Downer Grove, IL, USA) were, the LSI AML1/ETO Dual color probe Dual Fusion translocation probe to detect trisomy 8, LSI CSF1R (5q33-q34) SpectrumOrange/DS523, D5S721 SpectrumGreen probe to detect deletion of Chr 5q33-q34 region, LSI D7S486 (7q31) SpectrumOrange/CEP7 SpectrumGreen probe to detect deletion of Chr 7q31 locus and D20S108 (20q12) SpectrumOrange Probe to detect deletion Chr 20q12 locus. At least 200 interphase cells were scored for each probe.

Statistical analysis

Chi square test was applied for Statistical Analysis using the GraphPad software.

Results

Conventional cytogenetics was successfully carried out in 110 out of the 136 cases using GTG–banded metaphase analysis and chromosome aberrations were found in 40 of the 110 cases (36.4 %).

The FISH test yielded results in all the 136 cases studied and chromosomal aberrations were detected in 54 of these cases (39.7%). FISH and CC together identified an abnormal result in 70 of the 136 cases (51.5%) and male predominance was observed in the positive cases in the ratio of 2.5:1.

Figure 1 depicts the number of cases that showed a chromosomal aberration by either FISH or conventional cytogenetics, or both.

Comparison of CC analysis and FISH

Chromosomal aberrations were detected by conventional cytogenetics in 40 cases and these cases were further segregated into two groups. Group I is composed of nineteen cases, which included cases with at least one of the abnormalities namely –5/ –5q, –7/–7q, +8, –20q. The comparison of CC analysis and FISH on cases from Group I is summarized in Table 1.

Four cases showed chromosome aberrations by CC in addition to –5/ –5q, –7/–7q, +8, –20q. CC analysis showed –20q and –5q in Case No. 4 and Case No. 34 respectively, that were not detected by FISH. In Case No. 4, the metaphases revealed an additional chromosome 20 concomitant with –20q, leading to a normal signal pattern by FISH and hence was missed.

In Case No. 34, –5q was missed by FISH as the breakpoints of the deletion were (5q11.2-q13.1) which are farther from the most commonly deleted region (5q33–q34).

The FISH test could detect –20q in 3 cases, which were undetected by CC analysis, leading to an increment in the number of anomalies in these 3 cases (Case Nos 132, 124 and 117). Case No. 132 already had multiple anomalies, whereas in Case Nos 124 and 117, a single anomaly by CC was revised to a double anomaly by the additional information provided by FISH (see Table 1).

In Case No. 1, the FISH test identified duplication 7q and an atypical signal pattern indicating the presence of 4 copies of Chr 5p11.2 locus. Duplication of 7q was also observed by CC, however, no abnormality associated with Chr 5 could be detected. Instead, two novel translocations i.e. t(1;3;6) and t(10;20) were detected by the CC analysis. (Figure 2)

Group II consisted of twenty-one cases that showed chromosomal aberrations by CC excluding –5/ –5q, –7/–7q, +8, –20q. These cases are summarized in Table 2. Although these cases showed anomalies by CC analysis that were not within the scope of the FISH probes, FISH could detect a single anomaly in 4 cases (Case Nos 105, 97, 47, 104) and a double anomaly in 3 cases (Case Nos 135, 119, 114). Therefore, the cytogenetic profile of 4 cases changed from a single anomaly by CC analysis to a double anomaly by the inclusion of the FISH results. Similarly, 3 cases that were found to have a single