Hidden Chromosome 8 Abnormalities Detected by FISH in Adult Primary Myelodysplastic Syndromes

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Abstract. Acquired clonal chromosomal abnormalities are found in about 30-50% of primary myelodysplastic syndromes (MDS). These abnormalities are predominantly characterized by total/partial chromosomal losses or gains and rarely by balanced structural aberrations. Trisomy 8 represents the most common chromosomal gain. In the present study, the numerical aberration of chromosome 8 was evaluated by the fluorescence in situ hybridization (FISH) technique in MDS, and the results compared with those of conventional cytogenetics. Thirty adult patients with primary MDS, 17 with a normal karyotype and 13 with several chromosomal abnormalities except chromosome 8, were included in this study. On comparing the results of FISH and conventional cytogenetics, a superiority of FISH over the karyotype was detected in 3 cases. In one of them, further cytogenetic analysis confirmed the FISH results. Nevertheless, the FISH technique has limitations, detecting only abnormalities specific for the target FISH probe used. In clinical practice, conventional cytogenetics continues to be the basic technique for MDS patient evaluation. However, a large number of metaphases, even those of poor quality, must be analyzed in each case. The FISH technique could be considered to be complementary to achieve a more accurate analysis.

Acquired clonal chromosomal abnormalities are found in about 30-50% of adult primary myelodysplastic syndromes (MDS) and no specific cytogenetic abnormality is associated with a particular MDS French American British (FAB) subtype. These abnormalities are predominantly characterized by total/partial chromosomal losses or gains and rarely by balanced structural aberrations. Balanced aberrations, mainly translocations, have been shown to result in the production of fusion genes that are implicated in tumorigenesis, whereas gains or losses of genetic material may lead to a gene dosage effect or to the loss of tumor suppressor genes. Trisomy 8 represents the most common chromosomal gain, found in about 8-10% of cytogenetically abnormal MDS patients, while it is observed in all FAB subgroups (1-5). The significance of +8 as a risk factor in MDS is not well established. The International Prognostic Scoring System (IPSS) (6) ranked this abnormality in the intermediate-risk group, while other studies showed that +8 may carry a poor prognosis (7-9).

Conventional cytogenetics sometimes, due to technical difficulties, cannot be informative in MDS patient evaluation. With regard to numerical chromosomal aberrations, a number of studies have postulated the superiority of fluorescence in situ hybridization (FISH) over the karyotype. Using the FISH technique, a large number of cells can be examined and specific chromosomal aberrations can be detected in interphases as well as in metaphases (10-14).

In the present study, the numerical aberration of chromosome 8 was evaluated by the FISH technique in MDS and the results compared with those of conventional cytogenetics.

Materials and Methods

Thirty adult patients with primary MDS were included in this study, for whom a cytogenetic analysis by conventional cytogenetics had been performed. Clinical data for the patients were not available. As many cells as possible and not fewer than 15, were cytogenetically analyzed in each case. Seventeen patients had a normal karyotype, while in 13 chromosomal abnormalities were detected. Patients with chromosome 8 abnormalities were not included in this study. FISH was applied on recently made slides from the methanol/acetic acid-fixed cells of all patients, as described elsewhere (15). The α-satellite DNA probe D8Z2 specific for chromosome 8 (Cytocell Technologies, Cambridge, UK) (chromosome region 8p11.1 – q11.1) was used. The hybridization of the probe with the cellular DNA site was visualized by fluorescence microscopy NIKON E600 with a triple filter DAPI/FITC/TEXAS RED. Positive chromosome signals appeared as red spots in the nuclei. A minimum of about 150-200 cells from each slide were evaluated for each case. The signals were scored using the criteria of Hopman et al. (16). A case was counted as aberrant if more than 10% of the cell nuclei showed a loss or gain of signals for chromosome 8.

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Results
In all patients with a normal karyotype, the FISH results were in accordance with cytogenetic analysis. In two cases clonal trisomy 8 was detected by FISH (Figure 1). A re-evaluation of the cytogenetic analysis, by studying a larger number of metaphases, revealed in one of the patients with FISH abnormality two cells with trisomy 8. In the second case, there was no material for further cytogenetic analysis. Among patients with an abnormal karyotype, two presented cytogenetically an extra chromosome of unidentified origin. FISH analysis in one of them showed trisomy 8. In all the remaining cytogenetically abnormal patients, no FISH abnormalities of chromosome 8 were observed.

Discussion
The role of cytogenetic analysis in the diagnosis, prognosis and follow-up of MDS patients has been well defined. Several studies have shown the prognostic value of chromosomal abnormalities in predicting survival and risk of leukemic transformation during the MDS patient’s clinical course. Patients with single trisomy 8 according to the IPSS are in the intermediate-risk cytogenetic subgroup. However, several studies showed that patients with this abnormality have a significantly increased risk of leukemic evolution, suggesting that single trisomy 8 might be segregated from the intermediate-risk IPSS cytogenetic category (6-9, 17).

In clinical practice, because of the significant prognostic value of trisomy 8, it is important to detect MDS patients carrying this abnormality. Despite its importance, sometimes conventional cytogenetics, because of technical problems, cannot lead to an accurate analysis. The detection of clonal chromosomal abnormalities occurring in a minority of cells might be hampered by the poor quality of the metaphases. Moreover, due to nonrandom selection of the best metaphases, some chromosomal abnormalities occurring within bad-quality metaphases with overlapping chromosomes could be missed. Interphase FISH with specific probes provides a tool to study non-dividing cells, revealing numerical as well as structural chromosomal abnormalities, which could otherwise remain undetected.

This study was conducted to detect, by FISH, the presence of trisomy 8 in MDS patients, who presented either a normal or an abnormal karyotype but without chromosome 8 abnormalities. The FISH results were compared with those of conventional cytogenetics. Among 17 patients with a normal karyotype, two patients presented clonal trisomy 8. However, in one of them a further cytogenetic analysis with a larger number of analyzed metaphases confirmed the FISH results.

MDS patients with a normal karyotype constitute a heterogeneous group from the biological point of view. According to the IPSS, these patients have a good prognosis. However, a subset of them progress rapidly to acute myeloid leukemia. In such cases, hidden clones might be involved in the progression of MDS (18-22).

Among 13 of our patients with an abnormal karyotype, two presented an extra unidentified chromosome. Trisomy 8 was detected by FISH in one of them, suggesting that at least the pericentromeric area of chromosome 8 participated in the formation of this chromosome. All the remaining cytogenetically abnormal cases had two copies of chromosome 8.

Several studies have compared conventional cytogenetics and FISH in patients with a normal or an abnormal karyotype. They showed that sometimes an abnormal clone can be detected in patients with a normal karyotype, while in high-risk MDS patients, according to the IPSS, there is a greater probability for an abnormal karyotype to be detected by FISH. A good correlation of the two methods has also been described (18-21). However, the FISH technique has limitations. Thus,
chromosomal abnormalities involving any other chromosome or chromosomal region out of the specified FISH probe target can not be detected by the FISH technique. Thus, each method has its advantages and limitations, though in combination they may lead to a more accurate diagnosis, but cost/benefit must be taken into account.

In our study, a superiority of FISH over the karyotype was detected in three cases. In one of them further cytogenetic analysis confirmed the FISH results. Nevertheless, in clinical practice conventional cytogenetics continues to be the basic technique for MDS patient evaluation, detecting the overwhelming majority of cytogenetic aberrations, whereas it also has the potential to reveal chromosome anomalies that are not detected by most panel FISH tests used. However, a large number of metaphases in each case must be studied and an effort must be made to karyotype all the metaphases, even those of poor quality. The FISH technique can be used whenever conventional chromosome study is unsuccessful. Also, in cases with a suspected cytogenetic abnormality, the FISH technique is complementary, leading to an accurate evaluation.

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References


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