Diagnostic Role of a SNP Microarray in the Evaluation of Patients with Myelodysplastic Syndrome (MDS)

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Introduction

The diagnosis of neoplasia is complex and has been traditionally been augmented in the laboratory by cytogenetics, FISH and molecular analysis. However, many specimens are normal based on these tests, or often have incomplete results. Microarray analysis is now well established for the genetic evaluation of pediatric patients; however, its utilization for patients with neoplasia is still somewhat limited.

Objectives

The overall objectives of this work were to determine if SNP microarray could:

- Be utilized for the detection of abnormalities in chromosomally normal patients referred to determine if they had myelodysplasia
- Determine if it had a diagnostic component in patients in which flow cytometry and hematopathology studies could not conclusively make a diagnosis of MDS
- Delineate the presence of segmental uniparental disomy (UPD) and its implications
- Delineate the more common abnormalities detected by the array analysis and determine if these results could provide prognostic information
- Emphasize the importance of carefully considered guidelines for reporting abnormalities

Methods

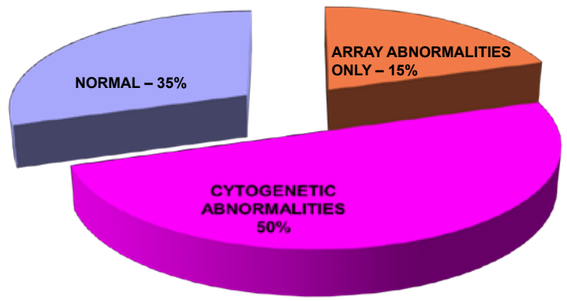
In order to determine the efficacy of this technology in MDS patients, a two phase validation study was undertaken, involving over 160 clinical specimens. In both parts of the study samples were evaluated initially with cytogenetics and/or FISH, and then by analysis with the Affymetrix SNP microarray analysis. Two different Affymetrix SNP platforms were utilized; the 6.0 version (with 1.8 million probes) in the initial evaluations, and the Cytoscan HD platform (with 2.695 million probes) in the latter part of the study. Criteria for reporting copy number changes were deletions greater than 1.0Mb and duplications greater than 2.0Mb. Deletions or duplications as small as 50kb were also reported in custom genes of known clinical significance. Possible UPD was reported when a homozygous block greater than 15Mb was present on a single chromosome interstitially and 8 Mb if involving telomeric regions. The initial study involved samples from patients evaluated because of a suspicion of MDS, both with and without chromosomal findings. The second phase of the study involved a group of low risk MDS patients who were evaluated by pathology and molecular technologies. In this group, neither morphology or flow cytometry could be utilized by a hematopathologist to always make a definitive diagnosis. These validation studies were then followed by the clinical evaluation of the array in which 440 patients were assessed by array analysis, complementing testing performed by morphology, flow cytometry, cytogenetic and FISH.

Results

Findings from the initial phase of the validation study:

In the initial study, our evaluation involved 114 patients that had been referred for routine chromosome studies and FISH, because of a suspicion of MDS, but with limited other clinical information provided. Approximately 50% of this referral group are detected to have a chromosome abnormality detectable by these techniques. Microarray analysis revealed that 30% of patients with a normal karyotype/normal FISH studies had a detectable abnormality by the array analysis. It also showed that in cytogenetically abnormal patients, numerous additional abnormalities could be detected by the array analysis.

Figure 1: Array results in initials MDS study



Findings from the second phase of the validation study:

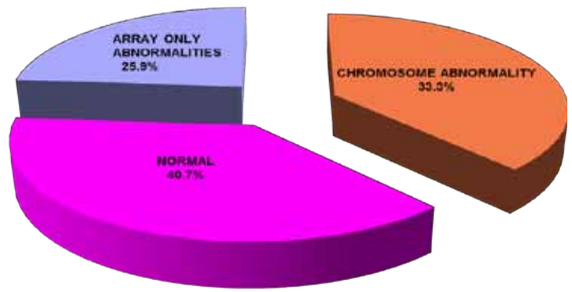
In the second phase of the validation study, a series of 48 patients were studied by cytogenetic/FISH analysis and then microarray analysis was utilized to determine if the information provided by the arrays could confirm a diagnosis. Twenty of the patients did not have MDS; with the other 28 considered to be low risk MDS patients. In 33.3% of the patients a chromosome abnormality was detected. In another 25.9% an array abnormality was detected, thus the array analysis was useful to assist in making a diagnosis in 62% of the patients.

Findings from the routine chromosome study:

Our initial evaluation studies clearly showed that the microarray technology is effective for studying patients with MDS. Therefore this has been added to our standard evaluation of patients when requested. Thus far we have studied over 450 patients and have correlated the array findings to other analysis in these patients including: chromosome and FISH studies; flow cytometry studies and hematopathology evaluation. These studies have shown the following:

- Overall, when chromosomes were normal, the array studies revealed an additional 24.3% of patients with abnormalities
- When chromosomes were abnormal, the array provided additional information 40% of the time and prognostic information in 36% of the patients, not available by the chromosome studies
- When both flow cytometry and hematopathology evaluation could not definitively confirm MDS, array analysis revealed abnormalities in 27.4% of the patients
- When either flow cytometry and hematopathology findings were consistent with MDS, array analysis revealed abnormalities in 80% of the patients
- 51.5% of the patients with abnormalities in this study had segmental UPD; the majority (54.9%)were as individual changes, while 39.2% were present with copy number changes
- Clonal evolution was seen in 21.2% of the patients and chromothripsis in 6.1%
- The most common changes seen involved the TET2 gene (19.2% – either as a deletion or UPD4q) and the RUNX1 gene (7.0%)

Figure 2: Array result from second validation study



Case example: An 89 year old male with pancytopenia was referred for evaluation for MDS. Initial cytogenetic and FISH studies were normal. Both flow cytometry evaluation (no aberrant lymphoid antigenic expression or monoclonality; monocytic findings - non-specific, but may be seen in association with early dysplasia) and hematopathology (trilineage hematopoiesis without increased blasts) could not conclusively provide a diagnosis of MDS. However array analysis showed normal dosage, but 20% mosaicism for long arm chromosome 4 allele homozygosity (segmental acquired uniparental disomy), suggesting a TET2 homozygous mutation.

Discussion and Conclusion

These findings have provided compelling results for the utilization of a SNP microarray for the evaluation of MDS. Using this technology, we are able to detect abnormalities in chromosomally normal patients even when flow cytometry and hematopathology studies couldn't effectively diagnose MDS. We were also able to clarify additional chromosomal anomalies and provide better prognostic information. In addition, SNP microarray technology provided for the detection of segmental UPD, which has been shown to be of extreme importance in MDS. With this delineation, we are able to identify candidate genes that may have mutations and importance in the cancer; these are localized in the homozygous blocks on the SNP microarray. Additionally, it is important to carefully consider guidelines for reporting abnormalities detected by the SNP microarray. In Oncology studies, obtaining complete clinical information, including previous karyotype and FISH findings, allows the lab to better interpret copy number and copy neutral changes identified by microarray. By obtaining complete clinical information, including previous karyotype and FISH findings, the lab is better able to interpret copy number and copy neutral changes identified by microarray. SNP microarray can be a useful tool in cancer analysis, and MDS in particular, for both patients with normal karyotypes or abnormal chromosome analysis.