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Utilization of magnetic activated cell sorting and high-density Single Nucleotide Polymorphism microarrays improves diagnostic yield and prognostic value in clinical testing for patients with multiple myeloma and normal routine chromosome study

Todd Christensen^a, Weiwen Deng^a, Bonnie McMahon^a, Joseph Schappert^a, Weihua Liu^a, Reza Saleki^a, and Ying S. Zou^{b,*}

^aPathology Associates Medical Laboratories, 110 W. Cliff Avenue, Spokane, WA.

^bDepartment of Pathology, University of Maryland School of Medicine, Baltimore, MD

Keywords

multiple myeloma; SNP microarray; cell sorting; loss of heterozygosity

Multiple myeloma (MM) is a clonal plasma cell disorder. Chromosome and FISH studies are used to provide prognostic information that is useful for refining risk stratification and therapeutic response [1]. Frequently, chromosome study is limited by the inability of plasma cells to proliferate in vitro, and FISH is restricted to small probe panels. To alleviate this problem, we have implemented a protocol of magnetic activated cell sorting (MACs) to isolate abnormal plasma cells followed by high-density single nucleotide polymorphism (SNP) microarray testing to improve our ability to clearly resolve chromosomal copy number abnormalities and to identify loss of heterozygosity (LOH) and copy number-neutral LOH (CNN-LOH). LOH/CNN-LOH have been shown to be important in cancer biology where they can lead to tumor suppressor gene inactivation [2]. By applying this methodology we have been able to increase our diagnostic yield and provide prognostic information on these clonal populations that previously would have been masked by the normal cells in the patient samples.

Three consecutive MM patients with enough bone marrow volume were used for this study. They had plasma cell ratios ranging from 16% to 77% based on morphology and from 4% to 20% based on flow cytometry (Table 1). All patients had normal karyotype results by chromosome studies. Interphase MM FISH panel including probe sets of D13S319/13q34, IGH/FGFR3, P53/CEP17, and MLL break-apart (Abbott Molecular) revealed only 1-2 anomalies per sample (Table 1). After MACs enrichment treatments (Stem Cell Technologies), SNP microarray was performed on both the plasma-enriched and the plasma-depleted fractions with a total of 2.6 million probes (Affymetrix CytoscanHD array). The

*Corresponding author: Ying S. Zou, MD, Ph.D., Bressler Research Bldg, Room 7025, University of Maryland Baltimore, 655 W., Baltimore St., Baltimore, MD 21201. Tel. +1 410-706-4063, Fax +1 410-706-6068, yzou@som.umaryland.edu.

microarray result of the plasma-depleted fraction was normal with no pathogenic deletions or duplications; however, microarray results of the plasma-enriched fraction revealed 8 - 12 chromosomal abnormalities. Follow-up FISH studies confirmed microarray findings using CBFB break-apart, LSI 1p36/1q25, BCR, LSI 21, and centromere probes (CEP) for X, 2, 3, 5, 7, 9, 11, 15, 17, 19 (Abbott Molecular) (Table 1) .

Microarrays were also performed on the total cell fraction of specimens from Patients 1 and 3 because of their high clonal plasma cell population (77% and 27% by morphology, respectively). By comparing microarray data between the total cell and plasma-enriched fractions of patients 1 and 3 (T and P+ in Table 1, respectively), it is evident that a more refined and accurate profile of chromosome copy number abnormalities is revealed from the plasma-enriched fraction than from the total fraction. For example, for chromosomes 5, 9, and 15 in patient 1, microarray revealed the presence of both trisomies and tetrasomies in the plasma-enriched fraction compared to only trisomies observed in the total cell fraction. Chromosomes 3, 7, 11, 17, 19, and 21 in patient 1 that were clearly trisomies in the plasma-enriched fraction dissolved into partial gain signals in the total cell fraction that were much more difficult to interpret with accuracy. For patient 3, microarray data from the total cell fraction only found the presence of mosaic loss of chromosome 13 and missed other eight additional changes, which were revealed by microarray data from the plasma-enriched fraction (Table 1).

In all cases, the prognostic information from the microarray of plasma-enriched fractions exceeded that of chromosome and FISH combined. According to microarray data, Patients 1 and 2 represent hyperdiploidy while Patient 3 is an example of hypodiploidy with 1q triplication that is also associated with a poor prognosis [3]. Besides identification of submicroscopic duplications or deletions, SNP microarrays can reveal abnormal allelic imbalances including LOH and CNN-LOH, which cannot be recognized by chromosome and FISH. CNN-LOH is the occurrence of LOH in the absence of allelic loss (copy number 2) and mosaic CNN-LOH is the mixture of normal and abnormal cells with CNN-LOH (Fig. 1a). Mosaic CNN-LOH of 16q was identified in Patient 2 (Fig. 1b), which has been associated with adverse prognosis [4]. Patient 3 was found to have complex abnormalities at chromosome 11q including mosaic deletion at 11q14.1-q22.1, a deletion at 11q22.1-q22.3, and mosaic CNN-LOH at 11q22.3-qter (Fig. 1c). Thus, plasma enrichment and SNP microarray provides a clear improvement in identification unbalanced chromosome abnormalities, LOH and CNN-LOH.

In summary, SNP microarray testing of MACs isolated abnormal plasma cells in our MM patients provided a much more comprehensive overview of the genome-wide chromosome copy number abnormalities compared to chromosome and FISH studies. SNP Microarray testing is useful in clinical practice to refine our diagnostic and prognostic indicators. This technique can be easily incorporated into every cytogenetic laboratory. Although SNP microarray testing cannot reveal any balanced structural abnormalities such as IGH-FGFR3 fusions, FISH on MACs isolated abnormal plasma cells will increase detection rate of clinically relevant genomic abnormalities to overcome the overall low percentage of plasma cells present in primary bone marrow aspirates. Therefore, combination of SNP microarray results of MACs enriched plasma cells and FISH/chromosome results will present a more

complete picture of chromosome abnormalities and provide insights into understanding mechanisms of MM formation and development.

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Figure 1.

Regions of CNN-LOH by SNP microarray. a Examples of different allele peak patterns determined by the ChAS software (Affymetrix). b Mosaic CNN-LOH at 16q in patient 2. c Complex 11q abnormalities in patient 3. del. = Deletion

Table 1
Summary of patients' clinical, karyotype (chromosome), FISH and SNP microarray data

	Patient 1	Patient 2	Patient 3
Clinical and karyotype data			
Clinical data	male, 48 years old	male, 72 years old	female, 92 years old
Plasma cells (morphology)	77%	16.5%	27%
CD38+/CD138+ plasma cells by flow-cytometric result	20% abnormal expression of CD56 and cytoplasmic κ light chain restriction	4% abnormal expression of CD56 and cytoplasmic λ light chain restriction	10% abnormal expression of cytoplasmic κ light chain restriction
Routine chromosome study	normal from short-term DSP30/IL-2-stimulated and unstimulated MarrowMax™ cultures	normal from short-term DSP30/IL-2-stimulated and unstimulated MarrowMax cultures	normal from short-term DSP30/IL-2-stimulated and unstimulated MarrowMax cultures
FISH and SNP microarray data			
Interphase FISH panel for MM	MLLx3 in 34.7% and TP53x3 in 23.3% normal for IGH/FGFR3 and D13S319/LAMP1	MLLx3 in 8.3% of cells normal for TP53, IGH/FGFR3 and D13S319/LAMP1	D13S319x1 and LAMP1x1 in 24% normal for TP53, IGH/FGFR3, MLL
ISCN based on hg19 of SNP microarray data	arr(3,7,11,17,19,21)×3, (5,9,15)×3-4	arr 1p31.1p21.1(76,346,199-100,161,575)×1.3p26.3q26.3(2,387,122-172,088,489)×3,(5,7,9, 11,15, 19,21)×3,16p13.3(351,832-735,154)×1,(2)×2 hnz,16q11.1q24(47,653,120-89,662,421)×2hnmz	arr (X)×1,q21.1q44(144,823,069-249,224,684)×4,(9)×3,11q22.1q22.3(99,871,813-103,082,590)×1,(13,22)×1,14q24.1q32.1(69,539,774-92,106,414)×1,11q22.3q25(104,660,736-133,796,675)×2 hnmz
SNP microarray results			
Total cell (T)	partial gain of chr. 3 13 trisomy 5 partial gain of chr. 7 trisomy 9 partial gain of chr. 11 trisomy 15 partial gain of chr.17 partial gain of chr.19 partial gain of chr.21 trisomy 3 (29%)	mosaic loss of chr.	gain of 1q21.1-q44 (19%)
Plasma-enriched (P+) (follow-up FISH results ^a)		mosaic CNN-LOH chr. 2	

Patient 1	Patient 2	Patient 3
trisomy 5 (17%)	partial trisomy 3 (gain of 3p26.3–q26.31 in 8%)	trisomy 9 (14%)
trisomy 7 (13%)		mosaic CNN-LOH 11q22.3–qter
trisomy 9 (17%)	trisomy 5 (9%)	monosomy 13 (24%)
trisomy 11 (28%)	trisomy 7 (11%)	monosomy 22 (16%)
trisomy 15 (16%)	trisomy 9 (8%)	monosomy X (15%)
trisomy 17 (22%)	trisomy 11 (9%)	mosaic deletion of 11q14.1–q22.1
trisomy 19 (21%)	trisomy 15 (16%)	deletion of 11q22.1–q22.3
trisomy 21 (19%)	mosaic CNN-LOH 16q11.1–qter	deletion of 14q24–q32.12
tetrasomy 5 (6%)	trisomy 19 (7%)	
tetrasomy 9 (9%)	trisomy 21 (10%)	
tetrasomy 15 (6%)	deletion of 1p21.1–p31.1	
	deletion of 16p13.3	

chr. = Chromosome; follow-up FISH results = CBBF, MLL, LSI 1p36/1q25, BCR, LSI 21 and centromere for X, 2, 3, 5, 7, 9, 11, 15, 17, 19 (Abbott Molecular); ISCN = an international system for human cytogenetic nomenclature.

^aConfirmation FISH after SNP microarray, percentages in total cells analyzed from primary bone marrow specimens.