

The Impact of FICTION on the Detection of Genetic Aberrations in Multiple Myeloma

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Abstract

Interphase fluorescence *in-situ* hybridization (FISH) has been employed to detect the genetic aberrations in multiple myeloma. FISH technique can increase the sensitivity for the detection of chromosomal aberrations, especially for the detection of cryptic genetic changes such as IgH/FGFR3 gene rearrangement of t(4;14)(p16;q32) translocation. However, FISH assay on unsorted cells resulted in falsely low positivity rate when non plasma cells (non-PCs) were also counted. Therefore, FISH after PC selected (either by CD138 sorting method or by FICTION technique) has been preferred.

FICTION technique allows the rapid identification of κ and λ positive PC within bone marrow by immunophenotyping and can simultaneously identify any specific genetic aberrations harboured by these cells. FICTION technique was characterized by rapid, convenience and specificity. Therefore, it was proved to be a useful tool in a clinical genetic laboratory for the detection of genetic aberrations in multiple myeloma.

Key words: FICTION, Fluorescence *in-situ* hybridization, cytogenetics, multiple myeloma, plasma cells

Introduction

Multiple myeloma (MM) is a B-cell

neoplasm in which, malignant plasma cells (PC) or clonal expansion of plasma cells accumulate in the bone marrow and the

malignant cells do not often move to the bloodstream from the bone marrow.¹ The production of a monoclonal immunoglobulin molecule (M protein) is a major characteristic of MM.² The M protein can be detected in the serum and/or urine. MM is a fatal and incurable disease that is included in about 10-15% hematological malignancies and 1% of adult malignancies.³ Long-lived PC is located at multiple sites and is associated with stromal cells in the bone marrow. A premalignant tumor named Monoclonal Gammopathy of Uncertain Significance (MGUS) often leads to MM.⁴ Less than 10% mononuclear cells in the bone marrow is found and the number of tumor cells remains stable unless the progression of MM.⁵ Based on the proliferation of monoclonal plasma cells in the bone marrow, the rate of proliferation of malignant plasma cells is an important determination of the disease biology. The disease remains incurable despite there are many advanced therapies. The exact cause of MM remains unclear, however, over-exposure to radiation will increase the risk of MM and the median survival is about 3-5 years.⁶

Conventional cytogenetic analysis

Large number of chromosomal and genetic abnormalities involved in MM has been detected in recent years. The most common genetic alterations are the deletion of chromosome 13q which associates with poor prognosis and gene rearrangement of immunoglobulin heavy chain gene (IgH) at 14q32.¹

Conventional cytogenetic (CC) analysis has been widely used to detect clonal chromosomal abnormalities of haematological malignancies. However, CC technique has limitation in detecting chromosomal abnormalities in MM patients and clonal chromosomal abnormalities are reported in only 30-40% of newly diagnosed cases.⁷ The reason for this is the extremely low proliferation rate of PCs, overall with less than 1% of cells synthesizing DNA, until late stages of MM.⁸ In addition, there are only limited PCs with bone marrow infiltration thus the obtaining of good metaphases for analysis is extremely difficult. Other reasons for the low frequency of chromosomal abnormalities are difficulties in obtaining spreads of good quality, burred and contracted chromosomes in direct harvest of bone marrow preparations,⁹ and a resistance of PCs to Giemsa-banding.¹⁰

Fluorescence *in-situ* hybridization assay

Interphase fluorescence *in-situ* hybridization (FISH), multicolor spectral karyotyping (SKY) and comparative genomic hybridization (CGH) have been employed to detect aneuploidy with chromosome abnormalities.^{4,11-14} The involvements of chromosome abnormalities in MM are structural change and/or complex numeric changes. Aneuploid karyotypes including trisomies (chromosomes 3, 5, 7, 9, 11, 15, 19 and 21) and monosomies (chromosomes 8, 13, 16, 17, 22 and Y) are frequently reported in previous literatures.^{4,11-14}

Structural rearrangements of IgH gene at 14q32, partial deletion of chromosome 13q, duplication of 1q, and deletion of 1p, 6q, 11q and 17p are commonly found in MM patients.^{4,7,14-16}

In view of the significant prognostic indicators, 13q deletion, rearrangements of IgH gene and deletion of TP53 are commonly investigated on MM patients in most clinical genetic laboratories. Locus specific identifier (LSI) FISH probes have been used to detect the presence, absence or the breakage of a particular involved gene sequence. All FISH probes used in the routine panel are commercially available. The classical panel includes LSI D13S319 (13q14.3) SpectrumOrange Probe (Vysis, Downers Grove, IL, USA) for the detection of the partial deletion of chromosome 13q. In addition, IgH SpectrumGreen/LSI FGFR3 SpectrumOrange Probe (Vysis) and TP53 SpectrumOrange/CEP 17 SpectrumGreen Probe (Vysis) can be employed to detect the IgH rearrangement and the loss of the TP53 region on the short arm of chromosome 17 respectively.

The principle of FISH is based on the hybridization of a DNA FISH probe to a complementary target gene DNA sequence. The probe is labeled directly with fluorescent dye. The probe and the target DNA are co-denatured at 75 °C and then hybridized together to let annealing of the complementary sequences to occur at 37 °C. After washing away the excess probes and the mis-matched annealed sequences at 73°C,

the fluorescence signals are visualized by using a fluorescence microscope.

Interphase FISH technique can increase the sensitivity for the detection of chromosomal aberrations in MM patients,¹⁷ especially for the detection of cryptic genetic changes, such as IgH/FGFR3 gene rearrangement of t(4;14)(p16;q32) translocation. Currently, IgH rearrangements are considered with high significance in prognosis in MM patients.^{16,18,19}

FISH assay on unsorted plasma cells

Cytospin slides are prepared from Carnoy's fixative fixed marrow cells and FISH is preformed according to manufacturer's instructions. FISH signals can be detected and evaluated using a fluorescence microscope, which is equipped with a sophisticated fluorescence imaging system for image processing. 100X oil immersion objective with a single-pass filter for SpectrumOrange, a single-pass filter for SpectrumGreen and a dual-pass filter for both SpectrumOrange and SpectrumGreen are used for slide examination. 200 cells are counted for the positivity of fluorescence signals and/or abnormal fluorescence patterns. The results are expressed as percentage of positive cells.

For the cells without 13q deletion, a pattern of two orange fluorescence signals (2O) should be observed. Loss of one of the orange signal (1O) should be shown in the cells with 13q deletion. For the cells without

t(4;14), a pattern of two orange and two green signals (2O2G), representing the two intact copies of the FGFR3 and IgH regions should be observed respectively. The cells with t(4;14), a fusion signal pattern presenting as a one orange one green and two fusion signals (1O1G2F) should be noted. A normal pattern of two orange and two green (2O2G) should be observed for the cells without TP53 deletions. For the cells with TP53 deletion, an abnormal pattern of one orange, two green (1O2G, i.e., loss of one TP53); one orange, one green signal (1O1G, i.e., monosomy 17); or two green signals (2G, i.e., loss of two TP53) etc. should be observed.

The disadvantage of FISH on unsorted cells is: false negative (falsely low positive) result usually obtained due to the low percentage of PCs presence in bone marrow. The non-PCs will also be counted and lead to a falsely low positivity rate.

FISH assay on CD138-purified plasma cells

Mononuclear cells from fresh marrow blood can be enriched by Ficoll-gradient centrifugation. An immunomagnetic cell selection procedure with anti-CD138 specific antibodies can be applied, using either the AutoMACS system (Miltenyi Biotec, Utrecht, The Netherlands) or CD138-coated magnetic beads (Easy Sep, Stem Cell Technologies, Grenoble, France) according to the manufacturer's instruction. Cytospin slides of the CD138 sorted cells are then

prepared and fixed with Carnoy's fixative. Further processing of slides, as well as scoring is identical as for FISH on unsorted slides. The flow chart for FISH assay on CD138-purified plasma cells was shown in Figure 1.

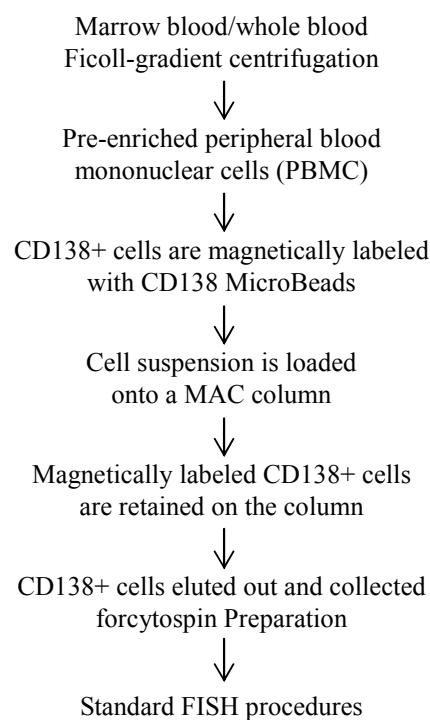


Figure 1. The flow chart of the preparation of CD138-purified plasma cells.

FICTION

In order to prevent the false counting of non-plasma cells, fluorescence immunophenotyping and interphase cytogenetic as a tool for the investigation of neoplasm (FICTION) has been introduced to investigate genetic alterations on PCs.^{20,21} In practical, it is the combination of fluorescence immunophenotyping and interphase FISH technique simultaneously on PCs. Firstly, FISH is performed

according to the standard protocol. Secondly, FITC-conjugated antibodies direct against human κ and λ light chain are used for staining the cytoplasm of PCs which allowed the positive identification of PCs. Finally, the scoring process includes a cytomorphological assessment to exclude non-PCs with false positive cytoplasmic immunostained - positive PCs.

Recently, we have successfully optimized the FICTION technique to identify the gene alterations in positively human κ and λ light chain (Dako, Enschede, Netherlands) stained PCs (Figure 2). FISH signals are scored only nuclei with one or more FISH signals of each probe. Based on our own experience, the cytoplasmic green fluorescence (labeled with FITC) is usually weak and the target gene signals will not be interfered with the green signals generated by FITC in the nucleus (Figure 3).

Conclusion

The clinical outcome of MM is heterogeneous, with survival ranging from a few months to more than 10 years.²² Genetic alterations in MM are correlated with prognosis.¹⁸ However, karyotyping fails to detect aberrations in 50-70% of the cases, probably because metaphases do not properly represent the malignant hematopoietic component.²³ In addition, some prognostically unfavorable chromosomal aberrations, such as: $t(4;14)(p16.3;q32)$ and $t(14;16)(q32;q23)$, can be cryptic in conventional cytogenetic analysis.

Interphase FISH can overcome this problem, and allowing to reveal cytogenetically cryptic aberrations.

To the best of our knowledge, FISH after PC selected (either by CD138 sorting method or by FICTION technique) has been preferred over FISH on unsorted cells because genetic abnormalities are more likely to be detected on selected PCs, especially when the number of investigated PCs increases. As there is a strong association between the detection rate of aberrations and the morphologically assessed PC infiltration, FISH on selected PCs should be the standard technique for the routine investigation of MM.

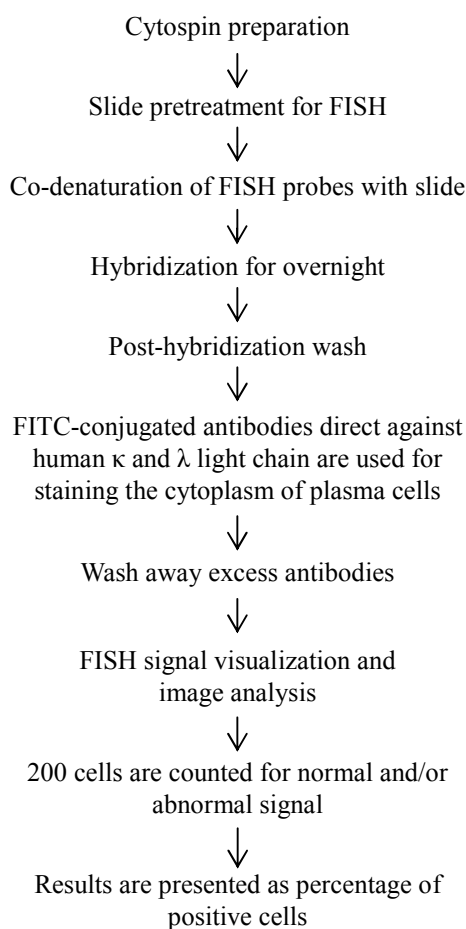


Figure 2. The flow chart of FICTION assay on plasma cells.

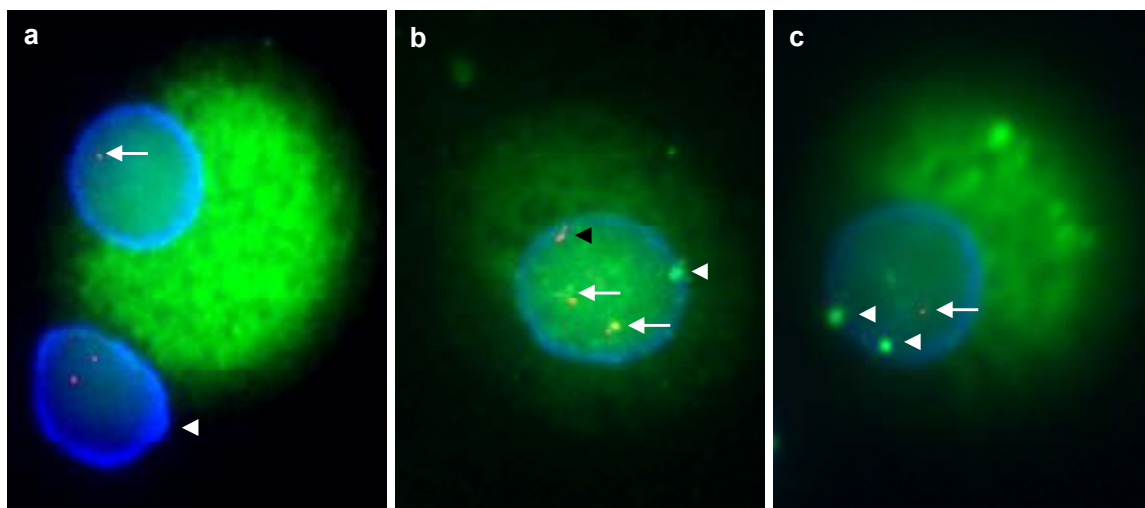


Figure 3. Detection of genetic alternations of plasma cells using FICTION technique and cytomorphological assessment. (a) Plasma cell stained with greenish cytoplasm with 13q14.3 deletion (arrow) and non plasma cell (negative greenish cytoplasm) with two 13q14.3 signals (arrowhead). (b) Plasma cell stained with greenish cytoplasm, 2 fusion signals (IgH-FGFR3 and FGFR3-IgH gene fusions, arrow), normal IgH green signal (white arrow head) and normal FGFR3 red signal (black arrowhead). (c) Plasma cell stained with greenish cytoplasm, 2 green chromosome 17 centromeric signal (arrowhead) and loss of one red TP53 signal on 17p (arrow).

CD138 sorting method is expensive and labor intensive, however FICTION technique is rapid, and technically easy to perform. Taken together, the FICTION technique is an indispensable tool in modern clinical genetic laboratory for the detection of genetic aberration in the diagnostic marrow of MM patients.

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