Multicolor interphase cytogenetics for the study of plasma cell dyscrasias

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Abstract. Specific chromosomal abnormalities such as chromosome 13 deletions and some translocations affecting the immunoglobulin heavy chain (*IGH*) gene, namely t(4;14)(p16;q32) and t(14;16)(q32;q23) have been associated with an adverse prognosis in multiple myeloma. Conventional cytogenetic techniques fail to detect these aberrations in the majority of cases. Thus, we have developed a novel set of interphase fluorescence *in situ* hybridization (I-FISH) assays targeting those regions frequently lost on chromosome 13 as well as those oncogenes most recurrently involved in translocations with the *IGH* locus in multiple myeloma, i.e. *IRTA1/2* (1q21), *FGFR3/MMSE*T (4p16), *CCND3* (6p21), *IRF4* (6p25), *CCND1* (11q13), *MAF* (16q23), and *MAFB* (20q12). The probes were combined in a multicolor fashion to develop novel multicolor I-FISH (MI-FISH) assays, whose validity and applicability was evaluated in negative controls and in a series of 13 plasma cell neoplasias. Additionally, a combination of the novel MI-FISH assays with staining for the plasma cell-specific antigen VS38c by means of multicolor FICTION (M-FICTION, fluorescence immunophenotyping and interphase cytogenetics as a tool for the investigation of neoplasms) allowed us to selectively analyze the plasma cell compartment, and thereby to increase the assay sensitivity.

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Introduction

Partial or complete losses of chromosome 13 and translocations affecting chromosomal band 14q32 containing the immunoglobulin heavy chain (*IGH*) locus have been reported to be the most common cytogenetic features in multiple myeloma (MM) and monoclonal gammopathy of unknown significance (MGUS) (1,2). Chromosome 13 abnormalities comprise mainly monosomy 13 (3) but also small interstitial deletions in chromosomal band 13q14 (4). These are detectable in 50-86% of cases (2,4,5). In contrast to many other B cell neoplasms, *IGH* translocations in MM affect a great variety of partner loci. The most recurrent *IGH* partners, together, present in ~40% of MM (6), are chromosomal bands 1q21 (*IRTA1/IRTA2*), 4p16 (*FGFR3/MMSET*), 6p21 (*CCND3*), 6p25 (*IRF4*), 11q13 (*CCND1*), 16q23 (*MAF*) and 20q12 (*MAFB*) (7). Several studies have suggested an association of chromosome 13 aberrations as well as some *IGH* translocations with unfavorable outcome both in patients treated with conventional or high-dose chemotherapy (8,9).

Due to the pathogenetic and prognostic impact of genetic aberrations in plasma cell dyscrasias (PCD) and in order to facilitate the diagnostic process, we developed and validated a new set of fluorescence *in situ* hybridization (FISH) probes for the detection of chromosome 13 abnormalities and for the most recurrent translocations affecting *IGH* loci in these diseases. We have shown the applicability of these probe sets for multicolor interphase (MI) cytogenetics. Moreover, by combining the novel MI-FISH assays with staining for the VS38c antigen according to the M-FICTION (multicolor-fluorescence immunophenotyping and interphase cytogenetics as a tool for the investigation of neoplasms) technique (10,11), we have provided a highly sensitive means for the simultaneous study of morphological, immunophenotypic and genetic features of PCD at the single-cell level.

Materials and methods

Patients and controls. Tumor samples from the bone marrow of 12 MM were analyzed in the present study. Eight of them were cytogenetically reported as normal. In one case (no. 3) no metaphases were obtained, whereas in another case (no. 4) no material was available for karyotyping. Cases no. 11 and 12 showed complex karyotypes. Additionally, the plasma cell leukemia (PCL)-derived cell line JJN3 was studied (12) (Table I). In 10 cases, we previously detected breakpoints affecting the *IGH* locus by means of double-color FISH using the LSI IGH break-apart probe (Abbott/Vysis, Downer Grove, IL, USA). Cytogenetic suspensions left from routine tumor cytogenetic analyses were used for MI-FISH analysis, whereas bone marrow smears were utilized for M-FICTION studies. All MM primary cases were retrieved from the cytogenetic database of the Department of Genetics of the University of Navarra in Pamplona, Spain, and the Institute of Human Genetics of the University Hospital Schleswig-Holstein Campus Kiel, Kiel, Germany. Peripheral blood mononuclear cells from healthy individuals with normal karyotype served as the negative controls. This study was performed within the context of the G03/136 Red temática FIS: 'Mieloma Múltiple y otras Ganmapatías', Spanish Ministry of Health, for which the approval of the Review Board was obtained.

Clone selection. Bioinformatic resources available at NCBI (http://www.ncbi.nlm.nhi.gov) and the University of California at Santa Cruz (version, May 2004) (http://www.genome.ucsc.edu) were utilized to select appropriate clones. All probes for the detection of *IGH* chromosomal rearrangements were designed in a way that they flanked the most recurrent breakpoints of the respective translocation described so far (Table II). The probes for the detection of chromosome 13 abnormalities were localized within the critical regions commonly deleted in PCD (Table III) (4). As internal control a probe for chromosome band 13q34 spanning STS marker D13S327 was selected.

Besides newly established probes, the LSI IGH dual-color break-apart rearrangement probe, LSI IGH-CCND1 dual-color dual-fusion translocation probe and LSI IGH-MYC-CEP8 triple-color dual-fusion translocation probe (all from Abbott/Vysis) were applied. For the detection of *CCND3* breakpoints a previously published FISH assay was used (13).

Probe preparation, FISH and multicolor FISH. Clones were cultured overnight in LB media supplemented with the appropriate antibiotic (chloramphenicol for BAC clones and kanamycin for PAC clones). DNA was prepared using the Perfectprep DNA Plasmid Maxi kit (Eppendorf, Köln, Germany). Clone DNAs were labelled with dUTP-Spectrum Orange (SO, Abbott/Vysis), dUTP-Spectrum Green (SG, Abbott/Vysis), dUTP-Texas Red (TR, Molecular Probes, OR, USA), dUTP-Cyanine5 (Cy5, Amersham Biosciences, UK) and dUTP-DEAC (NEN, Zaventem, Belgium) by random priming reaction (Bioprime, Life Technologies, Karlsruhe, Germany) exchanging the dNTP-Bio with the appropriate dNTP mixture. Labelled products were purified with Sephadex G-50 columns. Two hundred ng of each probe was coprecipitated with 5 μl of Cot1-DNA (Life Technologies) and re-suspended in 9 μl of master mix containing 2X SSC, 50% formamide and 10% dextran sulfate. Additionally, 1 μl of the proper commercial probe was added to the noncommercial probe mixture. FISH was performed as previously described (14). Slides were counterstained with DAPI and mounted in antifade solution. For dual-color and multicolor experiments, respectively, hybridization signals were analyzed by use of a Zeiss Axioskop 2 fluorescence microscope (Zeiss, Göttingen, Germany) with suitable filter sets (AHF, Tübingen, Germany) and documented using ISIS imaging system (Meta-Systems, Altlussheim, Germany) or a motorized fluorescence microscope (Axioplan 2 imaging mot, Zeiss) equipped with narrow band-pass filters and MetaSystems isis/mFISH imaging system (11).

Determination of cut-off levels for false-positive findings. For the determination of the diagnostic thresholds of the new probe sets, 200 nuclei were evaluated in each of five normal controls. In order to define precisely a 'split', the distance between the differently colored signals of a signal pair within the nucleus was estimated in relation to the signal diameter by visual inspection (14). The diagnostic cut-offs were calculated as the mean of false positives in the controls plus three times the standard deviation (SD) (15).

Multicolor FICTION (M-FICTION). For M-FICTION, staining with the monoclonal antibody (MoAb) VS38c that labels normal and neoplastic plasma cells (DakoCytomation, Glostrup, Denmark) was combined with the multicolor probe sets for the detection of *IGH* translocations and 13q deletions in PCD. Bone marrow smears were thawed and air-dried for 30 min at room temperature (RT), fixed in acetone for 10 min and air-dried. Slides were incubated for 30 min at RT with the primary MoAb anti-VS38c (1:20), diluted in PNM buffer (0.1 M $NaH_2PO_4 \cdot 2H_2O$ and 0.1 M $Na_2HPO_4 \cdot 2H_2O$).

Fluorescence detection was performed with a sequential cascade of three Alexa Fluor 350-conjugated Abs (Molecular Probes) diluted in PNM buffer (1:50) for 30 min each at RT; Alexa 350-conjugated rabbit anti-mouse; Alexa 350-conjugated goat anti-rabbit; and Alexa 350-conjugated donkey anti-goat. After immunophenotyping, slides were fixed in Carnoy's fixative (methanol:acetic acid, 3:1) for 10 min and in paraformaldehyde solution (1%) for 1 min. Then slides were dehydrated through increasing ethanol concentrations (70%, 85% and absolute) and air-dried. The multicolor probe pool (1.3 μl) was applied to the bone marrow smear and covered with a round 10-mm coverslip. Both probe and target DNA were simultaneously denatured at 70˚C for 7 min and incubated overnight at 37˚C. Post-hybridization washes were performed in 0.1X SSC three times for 5 min each at 60˚C. Then, slides were washed once in PN buffer and mounted in antifade solution. Image acquisition was performed using the same equipment cited above (11).

Results and discussion

Negative control studies. All probes (Tables II and III) hybridized to the correct chromosomal location in normal metaphases and gave two intense hybridization signals in

Table I. Clinical and cytogenetic features of the plasma cell dyscrasias studied by multicolor interphase cytogenetics.

Case no.	Age (years)	Gender	Karyotype	Interphase FISH results[a]			
				IGH break apart[b]	Oncogene fused to *IGH*	13q	Deleted region in 13q
1	69	Male	46,XY	nuc ish 14q32(*IGH*-cen x 2, *IGH*-tel x 1) [15%][e]	*CCND1*	nuc ish 13q(*RB1* x 2, D13S319 x 2, D13S25 x 2, D13S327 x 2)	None
2	67	Male	46,XY	nuc ish 14q32(*IGH*-cen x 2, IGH-tel x 1) [32%][e]	*CCND1*	nuc ish 13q(*RB1* x 2, D13S319 x 2, D13S25 x 2, D13S327 x 2)	None
3	82	Female	No metaphases	nuc ish 14q32(*IGH*-cen x 2, *IGH*-tel x 2) (*IGH*-cen sep *IGH*-tel x1) [15%]	*CCND1*	nuc ish 13q(*RB1* x 2, D13S25 x 2, D13S319 x 2 D13S327 x 2)	None
4	66	Male	ND	ND	*CCND1*[d]	nuc ish 13q(*RB1* x 2, D13S319 x 2, D13S25 x 2, D13S327 x 2)	None[d]
5	60	Female	46,XX	nuc ish 14q32(*IGH*-cen x 2, *IGH*-tel x 1) [30%][e]	*FGFR3*/ *MMSET*	nuc ish 13q(*RB1* x 1, D13S319 x 1, D13S25 x 1, D13S327 x 1) [30%]	Monosomy 13
6	74	Female	46,XX	nuc ish 14q32(*IGH*-cen x 2, *IGH*-tel x 2) (*IGH*-cen sep *IGH*-tel x1) [11%]	*FGFR3*/ *MMSET*	nuc ish 13q(*RB1* x 1, D13S319 x 1, D13S25 x 1, D13S327 x 1) [25%]	Monosomy 13
7	66	Female	46,XX	nuc ish 14q32(*IGH*-cen x 2, *IGH*-tel x 2) (*IGH*-cen sep *IGH*-tel x1) [9%]	*MYC*	nuc ish 13q(*RB1* x 2, D13S319 x 2, D13S25 x 2, D13S327 x 2)	None
8	63	Female	46,XX	nuc ish 14q32(*IGH*-cen x 2, *IGH*-tel x 2) (*IGH*-cen sep *IGH*-tel x1) [15%]	Not detected	nuc ish 13q(*RB1* x 1, D13S319 x 1, D13S25 x 1, D13S327 x 1) [27%]	Monosomy 13
9	72	Male	46,XY	nuc ish 14q32(*IGH*-cen x 2, *IGH*-tel x 2) (*IGH*-cen sep *IGH*-tel x1) [2%]	Not detected	nuc ish 13q(*RB1* x 2, D13S319 x 2, D13S25 x 2, D13S327 x 2)	None
10	37	Female	46,XX	nuc ish 14q32(*IGH*-cen x 2-3, *IGH*-tel x 1) [13%][e]	Not detected	nuc ish 13q(*RB1* x 1, D13S319 x 1, D13S25 x 1, D13S327 x 2) [27%]	*RB1*, D13S319, D13S25
11	68	Male	49,X,-Y,i(1)(q10)der(1)t(1;3)(q23;q13),+5,add(5)(q23), del(5)(q33),+7,+15/49, idem,dup(2)(p13p14)	nuc ish 14q32 (*IGH*-cen x 2, *IGH*-tel x 2)(*IGH*-cen sep *IGH*-tel x 1) [85%]	*IRF4*	ND	ND
12	79	Female	[c]45,XX,del(1)(p13),der(3), t(3;6)(p25;q?),der(6)t(X;1;6)(?;?;p25;q?),der(9)t(7;9)(q?;p11.2),-13t(14;20)(q32;q11.2)	ND	*MAFB*	ND	ND
JJN3 Ref. (12)	57	Female	58-67<3n>XX,+1,-2,+5,+8, +8,-9,-11,-12,-13,-15,-17, +20, add(1)(p22),der(1) t(1;?3)(q41;p26)x2,add(3)(p26), add(5)(p15)x1-2, i(5p),del(6)(q13),del(7)(q32), der(7)t(7;11)(q36;q13),add(8)(p1?)x2,t(12;19)(p13;q13)der(14)add(14)(p11) t(14;16)(q32;q23),t(14;16)(q32;q23),der(16)t(14;16)(q32;q23),del(22)(q12)	ND	*MAF*	ND	ND

[a]Described according to the ISCN-95 guidelines; cen, probe located centromeric to the gene; tel, probe located in the telomeric part of the gene; ND, not done. [b]LSI IGH dual-color, break-apart rearrangement probe (Abbot/Vysis). Cut-off level for false-positive findings was 1%. [c]Described by SKY FISH. [d]Detected by M-FICTION. [e]These FISH signal patterns may indicate the presence of an *IGH* translocation with loss of a derivative chromosome or a VDJ recombination affecting telomeric sequences of the V_H region.

Table II. Clones for the *IRTA1/2*, *FGFR3/MMSET*, *IRF4*, *MAF* and *MAFB* loci selected for the present study.

Chromosome band	Locus	Clone	Location to breakpoint	Start[a] (bp)	End[a] (bp)
1q21	*IRTA1/2*	RP11-110J1	Centromeric	153,785,333	153,952,624
		RP11-85G21	Centromeric	153,995,561	154,177,585
		RP11-217A12	Centromeric	154,198,015	154,333,751
	Breakpoint region (16)			*154,380,777*	*154,380,943*
		RP11-367J7	Telomeric	154,428,798	154,589,153
		RP11-120D12	Telomeric	154,589,054	154,652,686
		RP11-444M10	Telomeric	154,716,835	154,906,311
4p16	*FGFR3/MMSET*	CTD-3056L2	Centromeric	2,079,777	2,245,075
		RP11-1132M4	Centromeric	1,925,106	2,088,258
	Breakpoint region (17)			*1,824,392*	*1,872,576*
		RP11-572O17	Telomeric	1,592,782	1,782,006
6p25	*IRF4*	RP5-856G1	Centromeric	940,429	1,092,223
		RP5-1077H22	Centromeric	835,761	929,128
	Breakpoint region (18)			*140 kb 3' to IRF4*	*460 kb 5' to IRF4*
				IRF4 (start: 336,739	end: 356,442)
		RP3-416J7	Telomeric	89,702	213,735
16q23	*MAF*	RP11-571O6	Centromeric	76,153,174	76,329,694
		RP11-264M12	Centromeric	76,287,998	76,447,922
		RP11-281J9	Centromeric	76,434,195	76,606,528
	Breakpoint region (17,19)			*76,906,953*	*77,635,456*
		RP11-70D24	Telomeric	78,125,034	78,290,954
		RP11-345M22	Telomeric	78,289,994	78,477,920
20q12	*MAFB*	RP4-616B8	Centromeric	36,983,660	37,139,038
		RP3-404H4	Centromeric	37,410,145	37,566,011
	Breakpoint region (20)			*37,565,916*	*38,231,797*
		RP4-644L1	Telomeric	38,657,866	38,803,458
		RP1-94E24	Telomeric	38,886,168	39,028,514

[a]Position in base pairs according to UCSC Genome Browser (version, May 2004). Numbers in parentheses behind breakpoint regions indicate references.

interphase nuclei. Clones mapping centromeric and telomeric of the breakpoints of the *IRTA1/2*, *FGFR3/MMSET*, *IRF4*, *MAF* and *MAFB* loci were differentially labelled in SO or SG, pooled for each locus, and hybridized onto negative controls. Based on the signal patterns observed in these controls, a 'split' was uniformly defined for all of the break-apart assays as a spatial separation of signals flanking a given locus of more than three times the signal diameter as estimated by visual inspection. Using these criteria, the cut-off levels were 2.2% for *IRTA1/2*, 2.6% for FGFR3/MMSET, 3.0% for *IRF4*, 2.7% for *MAF* and 1.7% for *MAFB*. Clones within the commonly deleted regions in chromosome 13 were labelled in a multicolor fashion (Table III). Applying this probe set to normal controls, a cut-off was established for all signal constellations observed suggesting a deletion within the critical region in 13q14. The cut-off levels for all these patterns were <2%.

Multicolor interphase FISH: assay design. Due to technical considerations and previous experience we aimed to label each probe with a single dye rather than using combinatorial labeling, as this approach renders routine evaluation of the hybridization signals easier. The limitation of widely available fluorescence dyes restricted our labeling scheme to 6 colors (5 different fluorescent dyes plus DNA counterstain). Thus, we proposed a sequential, two-step procedure which took into account the prevalence of the different *IGH* translocations in PCD (Table IV). The first MI-FISH included probes for *FGFR3/MMSET*, *MAF* and *CCND3* in combination with the commercial probe LSI IGH/CCND1. This assay detects the most frequent and prognostically important *IGH* translocations in MM. The second MI-FISH assay included probes for *IRF4*, *IRTA1/2* and *MAFB* in combination with the commercial LSI IGH break-apart probe. The MI-FISH design is summarized in Table IV. A balanced rearrangement affecting *IGH* and any oncogene under study should normally lead to a double split and a double colocalization of the *IGH* probe with the locus-specific probe for the oncogene involved in the rearrangement (Fig. 1a, d and e). It is worth noting that in both MI-FISH assays, in addition to typical signal constellations, variant hybridization patterns including gains and losses of derivative and normal chromosomes as well as changes in ploidy may occur in interphase cells (Fig. 1b and c).

Table III. Selected clones spanning the RB1 locus and the STS markers D13S319, D13S25 and D13S327 for the present study and the fluorescent dyes used for the MI-FISH/FICTION PCD 13q assay.

Chromosome	Locus spanned	Start[a] (bp)	End[a] (bp)	Clone	Start[a] (bp)	End[a] (bp)	Fluorescent dye
13q14	*RB1*	47,775,911	47,954,023	RP11-305D15	47,760,579	47,897,921	SO
				RP11-174I10	47,897,822	47,960,646	
	D13S319	49,603,317	49,603,487	RP11-34F20	49,478,678	49,633,565	SG
				RP11-480P3	49,633,466	49,719,243	
				RP11-369L4	49,719,144	49,850,074	
	D13S25	50,143,128	50,143,379	RP11-175B12	49,973,084	50,099,510	DEAC
				RP11-58C16	*	50,191,416	
				RP11-233H19	50,187,167	50,328,618	
13q34	D13S327	114,040,208	114,040,462	RP11-245B11	113,770,458	113,932,864	TR
				RP11-569D9	113,930,806	114,103,243	

[a]Position in base pairs according to UCSC Genome Browser (version, May 2004). (*) Only one BAC end sequence is available.

Table IV. Sequential procedure for the detection of the most frequent *IGH* translocations in PCD.

	Probes	Fluorescent dye	Translocations
MI-FISH/FICTION PCD *IGH* assay 1	LSI IGH-CCND1 (Abbott/Vysis)[a]	SO and SG	t(11;14)(q13;q32)
	FGFR3/MMSET	TR	t(4;14)(p16;q32)
	MAF	DEAC	t(14;16)(q32;q23)
	CCND3	Cy5	t(6;14)(p21;q32)
MI-FISH/FICTION PCD *IGH* assay 2	LSI IGH break apart (Abbott/Vysis)[b]	SO and SG	t(14q32)
	IRF4	TR	t(6;14)(p25;q32)
	IRTA1/IRTA2	DEAC	t(1;14)(q21;q32)
	MAFB	Cy5	t(14;20)(q32;q12)

[a]The *IGH* locus probe was labelled in SG and the *CCND1* locus probe in SO. [b]The distal probe which covers the entire *IGH* variable region was labelled in SG. The proximal probe which lies 3' (centromeric) to the *IGH* locus was labelled in SO.

For the 13q MI-FISH assay (Table III), the loss of one signal from the probes spanning the *RB1* gene, D13S319 or D13S25 STS markers would imply a 13q14 deletion (Fig. 1i), whereas the signal pattern constellation indicating a monosomy 13 would also include loss of the signals derived from the clones spanning the telomeric internal control (STS D13S327) at 13q34 (Fig. 1h).

Due to the miniaturized hybridization format, it is, in principle, possible to perform up to 12 multicolor hybridizations on a single slide. Therefore, all three MI-FISH assays described here were performed simultaneously on a single slide (11). Moreover, a commercially available MI-FISH probe (Abbott/Vysis) for the detection of hyperdiploid MM, may be added (21).

MI-FISH in PCD with breakpoints at 14q32. In order to establish the diagnostic validity and applicability of our new *IGH* loci MI-FISH assays, we evaluated 12 primary MM and the PCL-derived cell line JJN3. Ten cases were previously shown to carry breakpoints affecting *IGH* loci by means of double-color FISH (Table I). Additionally, a t(14;20)(q32;q11.2) was detected by means of Spectral Karyotyping (SKY) in case no. 12 suggesting the involvement of the *IGH* loci. Similarly, the JJN3 cell line was previously reported to carry a t(14;16)(q32;q23) by means of G-banding analysis (12). Finally, bone marrow smears in case no. 4 were held in reserve for the M-FICTION assays.

In 8 out of the 12 MM, the partner gene involved in the *IGH* translocation was detected with the novel MI-FISH assays. Four cases, nos. 1-4 (case no. 4 detected by M-FICTION), displayed an aberrant signal constellation suggesting the presence of a t(11;14)(q13;q32) (Fig. 1c). Cases no. 5 and 6, showed a signal constellation indicating a t(4;14)(p16;q32) translocation affecting the *FGFR3/MMSET* genes (Fig. 1a). The PCL-derived cell line JJN3 displayed four fusions of the probes for *IGH* (SG) and *MAF* (DEAC) indicating the presence of a t(14;16)(q32;q23). Additionally, extra copies of the derivative chromosomes 14 or 16 and supernumerary signals for *CCND1*, *FRFG3/MMSET*, *MAF* and *IGH* indicated polyploidy (Fig. 1b). Cases no. 11 and 12 displayed the typical signal constellation suggesting the presence of t(6;14)(p25;q32) and t(14;20)(q32;q11)

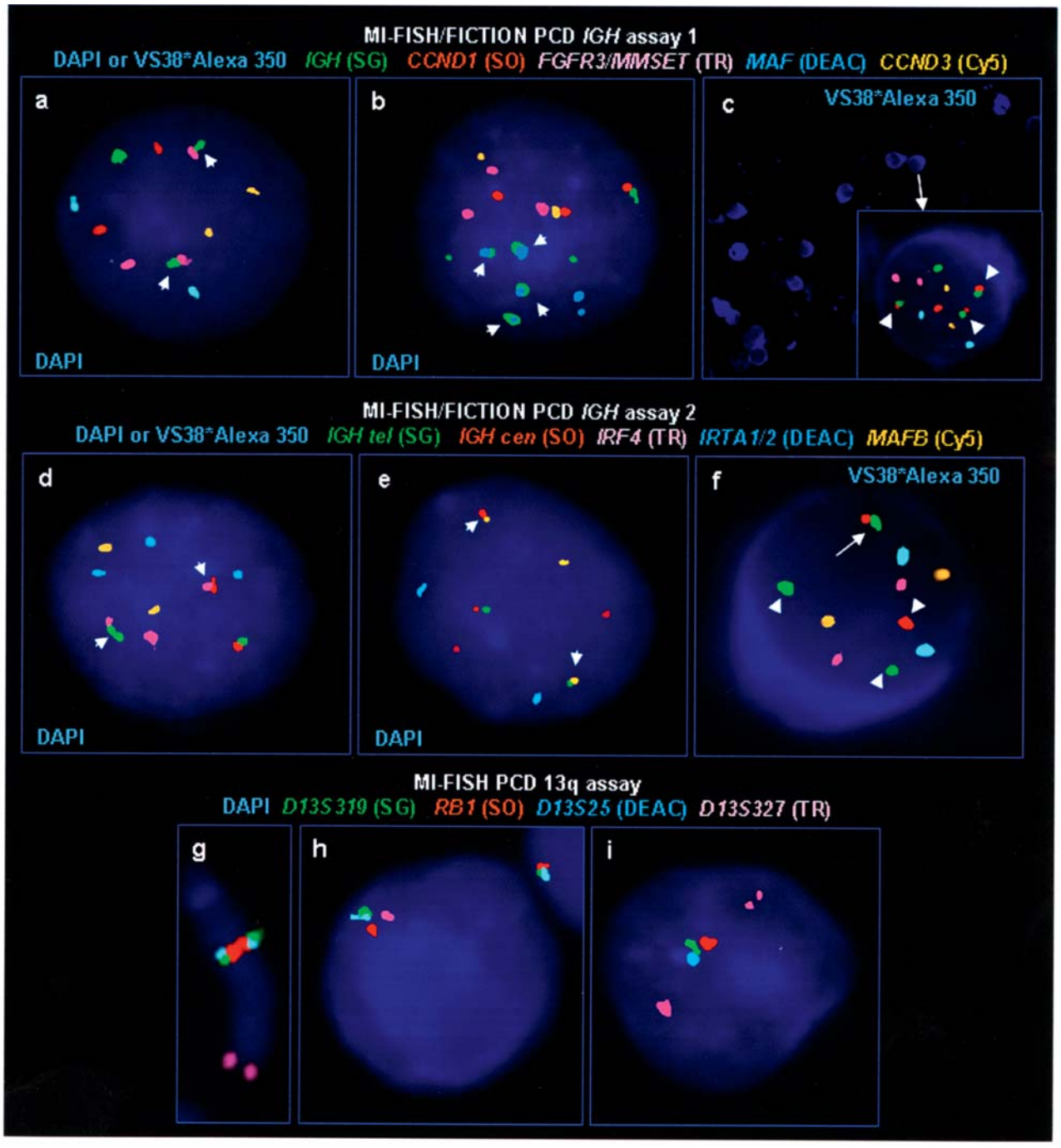

Figure 1. Multicolor interphase cytogenetics for the detection of *IGH* translocations and 13q deletions in plasma cell dyscrasias (PCD). Notice that MI-FISH and M-FICTION images are shown using a false-color display. FISH probes for the different PCD assays are color-typed according to their fluorescent staining (red scripts, SO labeling; green, SG labeling; light blue, DEAC labeling; pink, TR labeling; orange, Cy5 labeling; and dark blue, DAPI or Alexa fluor 350 staining). (a-c) MI-FISH/FICTION PCD *IGH* assay 1 include probes for *IGH*, *CCND1*, *FGFR3*/*MSET*, *MAF* and *CCND3*. (a) The interphase nucleus from case no. 6 shows a double fusion of the probes for *IGH* (SG) and *FGFR3*/*MMSET* (TR) indicating the presence of a t(4;14)(p16;q32). (b) The interphase nucleus from the JJN3 cell line shows four fusions (arrows) of the probes for *IGH* (SG) and *MAF* (DEAC) indicating the presence of a t(14;16)(q32;q23) plus an extra derivative chromosome 14 or 16 and additional signals for *CCND1*, *FRFG3*/*MMSET*, *MAF* and *IGH* loci, which might indicate polyploidy. (c) General overview of a bone marrow smear stained for VS38c, specific for plasma cells, from case no. 4. The indicated panel shows a VS38c-positive cell with plasma cell morphology displaying a triple fusion of the probes for *IGH* (SG) and *CCND1* (SO) indicating the presence of a t(11;14)(q13;q32) plus an extra derivative chromosome 11 or 14 (arrow heads). (d-f) MI-FISH/FICTION PCD *IGH* assay 2 includes probes for *IGH*, *IRF4*, *IRTA1/2* and *MAFB*. (d) The interphase nucleus from case no. 11 shows a double fusion (arrows) of the probes for *IGH* (SO-SG) and *IRF4* (TR) suggesting the presence of a t(6;14)(p25;q32) translocation, and one additional signal for *IRTA1/2* (DEAC). (e) The interphase nucleus from case no. 12 shows a double fusion (arrows) of the probes for *IGH* (SO-SG) and *MAFB* (Cy5) indicating the presence of a t(14;20)(q32;q12) translocation. (f) A VS38c-positive cell from case no. 4 shows a fusion of red and green signals which points to the intact *IGH* locus (arrow), whereas the split signals suggest the presence of a translocation affecting *IGH* (arrow head). The extra green signal indicates the presence of an extra derivative chromosome 14. This signal pattern is in line with the results obtained with the PCD IGH assay 1 shown in c. The *IGH* translocation corresponds to a t(11;14) as was shown applying the M-FICTION PCD IGH assay 1. Additionally, the M-FICTION PCD IGH assay 2 confirms the presence of an extra derivative chromosome, as was shown in c, and reveals that it corresponds to the der(11)t(11;14). (g-i) MI-FISH PCD 13q assay for the detection of chromosome 13 deletions including probes for the *RB1* gene, and the loci D13S319, D13S25 and D13S327. (g) Chromosome 13 from a normal control metaphase shows the regular signal constellation pattern. (h) Interphase nucleus from case no. 5 displays a signal constellation indicating monosomy 13. (i) The nucleus of case no. 10 displays a signal pattern indicating interstitial deletion in chromosomal band 13q14 with the loss of probes spanning *RB1*, D13S319, and D13S25.

translocations affecting *IRF4* and *MAFB* respectively (Fig. 1d and e).

The partner gene involved in the *IGH* translocation in the other four cases remained unidentified by the novel MI-FISH assays. One of the cases (no. 7) was subsequently shown to carry an *IGH-MYC* fusion. The lack of identifiable *IGH* partners in the remaining cases was most likely due to the great number of loci involved in *IGH* rearrangements in MM. All had a prevalence of ≤1%, and therefore were not included in the MI-FISH assays described here (7).

MI-FISH for the detection of 13 deletions in PCD. All 10 MM (nos. 1-10), for which conventional cytogenetics did not show an aberrant karyotype, were investigated with the 13q MI-FISH assay (Table I).

Three out of ten (nos. 5, 6 and 8) displayed a signal constellation indicating the loss of a complete chromosome 13 (Fig. 1h). Case no. 10 displayed a signal constellation pointing to an interstitial deletion of chromosomal band 13q14 with the loss of the *RB1* gene, and the markers D13S319 and D13S25 (Fig. 1i).

M-FICTION in PCD. Unstained bone marrow smears were available only for case no. 4 in this series. M-FICTION was applied using the same multicolor probes in combination with the plasma cell-specific MoAb VS38c. The results clearly showed that *IGH-CCND1* fusion was present exclusively in the plasma cell subset (VS38c-positive, Fig. 1c). A deletion affecting 13q was not found in this case. Plasma cells without *IGH* alterations existed as well, most likely representing the nonmalignant plasma cell population. All evaluated cells lacking VS38c staining displayed normal signal constellations.

In conclusion, the detection of *IGH* translocations and chromosome 13 deletions is important for the diagnostic and the clinical management of patients with PCD (8,9). Recently, Wuilleme and colleagues showed that the MI-FISH approach is a sensitive method for the detection of hyperdiploidy in MM regardless of metaphase availability (21). Additionally, commercial suppliers have released some reliable probes for the detection of the most frequent chromosome aberrations in MM, such as del(13q)/-13, t(11;14), t(4;14) and t(14;14). However, these commercial probes are only available for a limited number of *IGH* translocation partners and are not yet available as multicolor diagnostic sets.

Therefore, we designed a set of FISH probes for the detection of *IGH* translocations and 13q deletions. We have shown the applicability of this probe set for multicolor interphase cytogenetics by means of MI-FISH and M-FICTION. However, due to the versatility of the probe design, it was also possible to label them in a dual-color fashion in order to detect chromosomal breakpoints in a gene regardless of its partner chromosome by a break-apart approach, or to combine them with other commercially available or self-made FISH probes, e.g. for the *IG* light chain loci.

Thus, with these novel MI-FISH and M-FICTION assays, we have provided new reliable tools for the rapid and sensitive detection of those chromosomal abnormalities with prognostic implications affecting the *IGH* loci and chromosome 13 in the plasma cell compartment and at the single-cell level. These novel means for the cytogenetic analysis of plasma cell neoplasms may be applied for initial characterization, evaluation of follow-up and monitoring of minimal residual disease in plasma cell dyscrasias.

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