VARIANT *IGH* FISH PATTERNS IN PLASMA CELL MYELOMA

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ABSTRACT

Rearrangements involving the immunoglobulin heavy (*IGH*) locus at chromosome 14q32 are frequent in B-cell malignancies, specifically lymphoma and plasma cell myeloma patients. *IGH* gene rearrangements typically lead to deregulated gene expression of the *IGH* translocation partner genes, and therefore the identification of these *IGH* gene rearrangements and their translocation partner can help with patient risk stratification and therapeutic options. Interphase FISH analysis utilizing an *IGH* break-apart (BAP) probe design is typically used in the FISH laboratory to detect rearrangements of *IGH* and if positive, a reflex FISH panel is performed with *IGH* dual fusion probe sets including *FGFR3* (4p16.3), *CCND1* (11q13), *MAF* (16q23), and *MAFB* (20q12).

The classic break-apart *IGH* rearrangement signal pattern of 1R1G1F is well documented in the literature, so the purpose of this study was to scrutinize atypical patterns. A series of 300 cases including any abnormal *IGH* break-apart signal patterns such as 1R1F, 1G1F, and red and/or green signals without a normal fusion were investigated to resolve if the atypical patterns were associated with a gene rearrangement versus somatic recombination. Cases with these deletion patterns were reflexed to question an *IGH* translocation partner.

IGH aberrations were classified into seven groups including 1) Typical rearrangement, 2) 5’ deletion, 3) 3’ deletion, 4) Combination of typical and deleted patterns, 5) Red and green signals without normal homologue, 6) Complete gains of *IGH*, and 7) Complete loss of *IGH*. The normal (2F) signal pattern was identified in 45% of cases. The abnormal groups involved the classic 1R1G1F signal pattern as a sole abnormality in 13% of the cases, while the remaining 6 abnormal groups contained 42% of the cases. Implications of atypical *IGH* gene break-apart patterns concerning aberration mechanisms, incidence of translocation partners, prognosis, and therapeutic options will be discussed.

BACKGROUND

Results with BAP *IGH* probes are varied including the expected typical rearrangement pattern (1R1G1F), and frequently, 5’*IGH* (*IGHV*) deleted patterns (≥1R≥1F), 3’*IGH* (*IGHC*) deleted patterns (≥1G≥1F), and depending on probe vendor, partially deleted 5’*IGH* (diminished signal) patterns (1dim green annexed to a red signal), see figures 1 & 2. BAP *IGH* patterns suggestive of loss 3’*IGH*, loss of 5’*IGH*, or partial loss of 5’*IGH* can be ambiguous in interpretation and in impetus to reflex to a dual-fusion (D-FISH) panel. *IGH* deletions of varying size, genomic location, and zygosity have been documented in B-cell malignancies, particularly in chronic lymphocytic leukemia, diffuse large B-cell lymphoma, B-cell acute lymphoblastic leukemia, and to a lesser extent, plasma cell neoplasms. Deletions can be commonly attributed to secondary events following any chromosomal rearrangement (unbalanced translocations). However, some studies caution interpretation of 5’*IGH* (*IGHV*) deletions citing that certain cryptic deletions of the *IGHV* region may be the result of the DNA loss that accompanies normal somatic VD-J recombination and may not have any oncogenic implications in B-cell malignancies.

MATERIALS AND METHODS

To aid in the interpretation of clinical cases with partial *IGH* deletions, our group sought to compare 1) the incidence of *IGH* typical rearrangement vs. rearrangement and/or partial *IGH* deletion, 2) quantify the frequency of the involved gene translocation partners, and 3) speculate deletion mechanisms in a retrospective series of 300 plasma-cell-enriched (CD138+) bone marrow specimens referred to NeoGenomics Laboratories for plasma cell myeloma. The series was initially evaluated with a Plasma Cell Myeloma panel consisting of enumeration probes for chromosomes 1p/1q, 5, 9, 13q, 15, 17p, and a BAP for *IGH* and included samples with normal BAP *IGH* and samples with any BAP *IGH* abnormality. Cases with typical *IGH* rearrangement (1R1G1F) and those with partial *IGH* deletion(s) were reflexed to a D-FISH panel comprising *FGFR3* (4p16.3), *CCND1* (11q13), *MAF* (16q23), and *MAFB* (20q12).

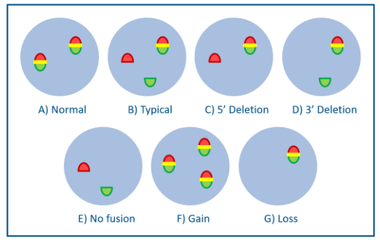

FIGURE 1. Common patterns observed with BAP *IGH*. A) Normal-2F **B)** Typical abnormal-1R1G1F **C)** 5’*IGHV* deletion-1R1F **D)** 3’*IGHC* deletion-1G1F **E)** Loss of normal homologue (fusion)-1R1G0F **F)** Complete gain of *IGH* **G)** Complete loss of *IGH*

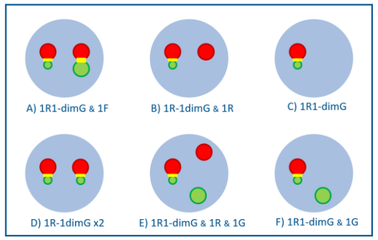

FIGURE 2. Permutations of *IGHV* partial deletions. A) 5’ *IGH* partial deletion & normal *IGH* **B)** 5’ *IGH* partial deletion & complete 5’ *IGH* deletion **C)** 5’ *IGH* partial deletion & 3’ and 5’ *IGH* deletion **D)** 5’ *IGH* partial deletion on both homologues **E)** 5’ *IGH* partial deletion & *IGH* separation **F)** 5’ *IGH* partial deletion & 3’ *IGH* complete deletion

	BREAK-APART *IGH*						D-FISH CHROMOSOME PARTNER				
	CATEGORY	PRINCIPAL PATTERN	ANCILLARY PATTERNS	INCIDENCE	CASES	FUSION POSITIVE	MODAL PATTERN	4 *(FGFR3)*	11 *(CCND1)*	16 *(MAF)*	20 *(MAFB)*
1	TYPICAL	1R1G1F	NONE	13.0%	39	80%	--	33%	57%	7%	3%
2	5' deletion	1R1F	2R1F, 2R2F, 2R	19.3%	58	78%	2R1G1F	9%	82%	4%	4%
3	3' deletion	1G1F	2G1F, 1~2G2F	6.3%	19	68%	1R1G1F	100%	0%	0%	0%
4	TYPICAL + ATYPICAL	1R1G1F	R, G, NO FUSION	9.0%	27	82%	--	19%	67%	10%	5%
5	NO FUSION	1R1G	2R2G, 1R2G, etc	3.7%	11	82%	--	44%	22%	22%	11%
6	GAIN	3F	4F	1.3%	4	--	--	--	--	--	--
7	LOSS	1F	NONE	2.3%	7	--	--	--	--	--	--
	NORMAL	2F	2F	45.0%	135	--	--	--	--	--	--

TABLE 1. Incidence of *IGH* aberrations in 300 plasma-cell-enriched samples and their translocation partners.

RESULTS AND DISCUSSION

Probes in the Plasma Cell Myeloma panel detected recurrent variations of chromosomal gains of 1q, 5, 9, 11, 15 and losses of 1p, 13, and 16 (not reported here) in samples with and without *IGH* abnormalities. Normal BAP *IGH* (2F) was observed in 45% of all cases. BAP *IGH* aberrations were classified into seven categories based on their signal patterns, see Table1.

1) Typical: Only 13% of cases had the typical (1R1G1F) separation pattern as a sole abnormality, and of these, 80% were positive for the identification of a translocation gene partner. The principal partner in this group was *CCND1* (57%), followed by *FGFR3* (33%).

2) 5’ deletion group, with loss of green signal(s), had an incidence of 19% with positivity of 78% and *CCND1* (82%) as the principal partner.

3) 3’ (red) deletion was represented in 6% of cases with a positivity of 68% and *FGFR3* notably as the exclusive translocation partner.

4) A combination of the typical and deleted patterns, engaged 9% of cases, had a positivity rate of 82%, and the most common partners were *CCND1* (67%) and *FGFR3* (19%).

5) Patterns of red and green signals without a normal homologue were rare at <4% and translocated 82% of the time with mixed partners: *FGFR3* (44%), *CCND1* (22%), *MAF* (22%), and *MAFB* (11%).

6&7) Complete gains and loss of *IGH* (3’ and 5’) was identified in 1.3% and 2.3% of cases, respectively.

The modal fusion signal pattern for 3’*IGH* deletion with fusion to *FGFR3* was 1R1G1F, and for 5’*IGH* deletion with fusion to *CCND1* it was 2R1G1F when reflexed to the respective D-FISH probe sets. These findings suggest that the first represents loss of the derivative chromosome 14 after reciprocal translocation with chromosome 4, and the second implies an interstitial deletion including 5’*IGH* following rearrangement rather than loss of the derivative chromosome 14 (Figure 3).

A separate group of cases was observed anecdotally to display an incomplete deletion of 5’*IGH*, i.e., a minute green signal remained annexed to one or both of the red signals, figure 2. A translocation partner was not identified for any of the 43 cases with partial *IGHV* deletions. These 5’ diminished signal patterns were observed using Abbott’s break-apart probes, but may not be detected by probes from all manufacturers. A probe design to avoid detecting these partial deletion patterns for the sake of interpretation simplicity may be with purpose. However, this set of cases may harbor a deletion of specific size and location exclusively associated to the described *IGHV* deletions due to somatic recombination.

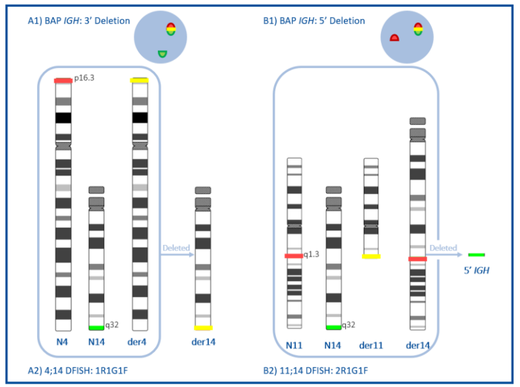

FIGURE 3. Proposed mechanisms for unbalanced rearrangements in two unrelated samples as revealed by BAP and D-FISH correlation. **A1)** BAP *IGH* resulted 1G1F. **A2)** The t(4;14) probes revealed signals for a normal 4, normal 14, and a derivative 4 (1R1G1F), representing loss of the derivative chromosome 14 containing 3’*IGH* after reciprocal translocation with chromosome 4. **B1)** BAP *IGH* resulted 1R1F. **B2)** The t(11;14) reveled signals for a normal 11, derivative 14, normal 14, and a derivative 11 (2R1G1F, respectively), representing an interstitial deletion including 5’*IGH* rather than loss of the derivative 14.

CONCLUSIONS

Atypical signal patterns are more common than the classic abnormal signal pattern in plasma cell myeloma cases when investigated with a BAP *IGH* probe set. Cases demonstrating a 3’ or 5’ *IGH* deletion, as with cases with the typical break-apart signal pattern, should be reflexed to a D-FISH set of probes since the majority will have an identifiable translocation partner. The detected BAP *IGH* category can help predict the likelihood of a specific gene partner and thus guide D-FISH probe selection hierarchy, especially when an enriched sample is limited. The presence and size of a 5’ diminished signal, with use of a specifically designed and tested probe set, could serve as a tool to differentiate cases with oncogenic *IGH* juxtaposition due to an unbalanced rearrangement from those exhibiting deletions caused by benign somatic *IGHV* recombination, for which a reflex study is not necessary.

REFERENCES

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