IGH rearrangement in myeloid neoplasms

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Supplementary Information

Materials and Methods

Karyotype

Cells from bone marrow aspirate in sodium heparin were counted with a TC-20 Automated Cell Counter (Bio-Rad, Hercules, CA). 5×10^6 of cells were cultured in Marrow Max (Life Technologies, Carlsbad, CA) or Chang Marrow (Irvine Scientific, Santa Ana, *CA*) for 15-24 h. Cells were treated with ethidium bromide (5 μg/mL) for 1.5 h and colcemid (0.5 μg/mL) for 20 min, incubated in a hypotonic solution, fixed, and metaphase spreading slides were manually made. After baking at 70ºC for 1 h, chromosomes were Giemsa-Trypsin banded and analyzed.

Fluorescence in Situ Hybridization (FISH)

Fresh-made metaphase slides were incubated in 2×SSC at 37℃ for 30 min and dehydrated in 70% 80%, 90%, and 100% ethanol. 10 μl of probe was added to a slide, denatured at 80℃ for 1 min, and hybridized at 37℃ overnight in a ThermoBrite™ hybridizer (Thermo Fisher Scientific, Carlsbad, CA). Post-hybridization wash was done in 0.4xSSC/ 0.3% NP-40 at 73℃ for 3 min and slides counterstained with DAPI. For re-hybridization, slides were stripped/denatured in 70% Formamide at 73℃ for 2 min and dehydrated in ethanol solutions. Probes were denatured at 73℃ for 5 min before adding to the stripped slides for hybridization.

Mate-pair next-generation sequencing (NGS)

DNA was extracted from leftover cytogenetic cell pellet with a standard proteinase K and SDS protocol. DNA was processed using the Illumina Nextera Mate Pair library preparation kit (Illumina, San Diego, CA) and was sequenced on the Illumina HiSeq 2500 in rapid run mode as previously described [1,2,3]. Pooled libraries were then hybridized onto a flow cell and sequenced using 101-basepair reads and paired end sequencing at the Mayo Clinic Medical Genome Facility. Sequencing data were mapped

to the reference genome (GRCh38) using the BIMA V3 alignment algorithm and then analyzed using SVAtools [2,4], which is used to detect the breakpoint locations of structural variants and copy number abnormalities. Abnormalities are graphically illustrated using genome, junction, and region plots as previously described in Aypar, et al[1].

Realtime quantitative RT-PCR

Total RNA was isolated from unstained bone marrow aspirate smears using the TRIZOL reagent according to the manufacturer's instructions (Thermo Fisher Scientific). The RNA integrity was evaluated in agarose gel electrophoresis. A real-time quantitative RT-PCR was performed using iTaq Universal SYBR Green One-Step Kit (Bio-Rad). 200 ng of total RNA was added to PCR reactions containing iScript reverse transcriptase, SYBR Green, iTaq DNA polymerase, and *CCNG1*- and *ACTB*-specific primers. The reactions were incubated at 50^{o}C for 10 min for reverse transcription and subjected to 40 cycles of 95^{o}C for 10 sec and 62^{o}C for 60 sec performed on an ABI Prism 7700 Sequence Detection System (Applied Biosystems, Carlsbad, CA). The expression level of the *CCNG1* was normalized to *ACTB*. All experiments were performed three times with comparable results.

This study is approved by the institutional review board at Brigham and Women's Hospital and Mayo Clinic (case 1).

References

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Tab. 1: Primers used for *IGH-CCNG1* fusion confirmation. Base-stacking calculation is used for melting temperature (Tm) estimation.

Primer name	Primer sequences	Tm	Size (bp)
	1st PCR		
IGH_F fusion	CATTACTCCCAATAGCATATTCTCAG	62.2	1230
CCNG1_R fusion	CCTGATGTAGAGCCAGAGAATAAG	63.1	
	2nd PCR		
IGH_F fusion nest	CTCCATGTTAATTTATAATAGTGGCATC	61.6	1172
CCNG1_R fusion nest	CTGTGGCTGCCTATCCAG	62.7	

Tab. 2: Primers used for *CCNG1* realtime quantitative RT-PCR. Base-stacking calculation is used for melting temperature (Tm) estimation.

Primer name	Primer sequences	Tm	Size (bp)
CCNG1_F qPCR	AATGAAGGTACAGCCCAAGCA	65.2	239
CCNG1_R qPCR	GCTTTGACTTTCCAACACACC	63.3	
ACTB_F qPCR	AGAGCTACGAGCTGCCTGAC	67	184
ACTB_R qPCR	AGCACTGTGTTGGCGTACAG	66.1	