Chromosome 6q deletions are common in Waldenström's Macroglobulinemia, and target regulatory genes for MYD88, CXCR4 and BCL2 signaling

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BACKGROUND

Activating mutations in MYD88 mutations are highly prevalent (>90%) in Waldenström's Macroglobulinemia (WM), and trigger pro-survival NFkB signaling. Activating mutations in CXCR4 are also present in 30-40% of WM patients, trigger pro-survival AKT and ERK1/2 signaling, and are associated with both in vitro and clinical resistance to ibrutinib. Deletions involving the long arm of chromosome 6 (chr. 6q) are a common abnormality in WM, and include genes that modulate NFkB activity (TNFAIP3, HIVEP2, IBTK), BCL2 family of proteins (BCLAF1), apoptosis (FOXO3), and BTK (IBTK), the target of ibrutinib. The impact of chr. 6q deletions on the expression of these critical survival determining genes remains unclear in WM.

AIMS

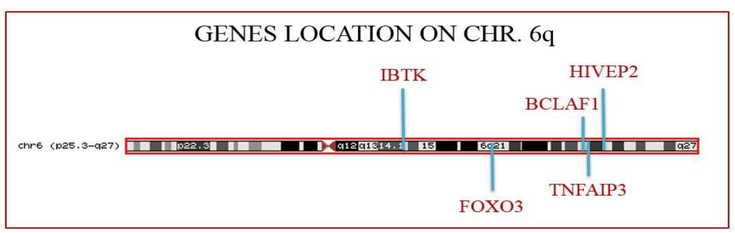
Whole genome sequencing studies previously demonstrated that focal deletions can cause the gene deletion rate to vary significantly across the q arm of chromosome 6 (Hunter et al Blood, 2013). Many deletions were found to exist only within a WM subclone. The impact of these 6q deletions on the expression of genes critical to WM survival remain unclear. The aim of this study is to investigate potential target genes and to study the effect of their deletion on gene expression and disease phenotype.

PATIENTS AND METHODS

CNAs were measured in quadruplicate and gene expression in triplicate using TaqMan real-time polymerase chain reaction (RT-PCR) assays. DNA and RNA from CD19+ sorted bone marrow lymphoplasmacytic cells from 24 untreated WM patients and 1 patient with IgM and IgG-secreting lymphoplasmacytic lymphoma (LPL) were analyzed (Patient characteristics are listed in Table 1). Paired CD19-depleted peripheral-blood mononuclear cells (PBMCs) were used as germline controls. Paired CD19+ and CD19- PBMCs from 6 healthy donors were used to rule out possible B-cell specific findings.

TABLE 1: PATIENT CHARACTERISTICS

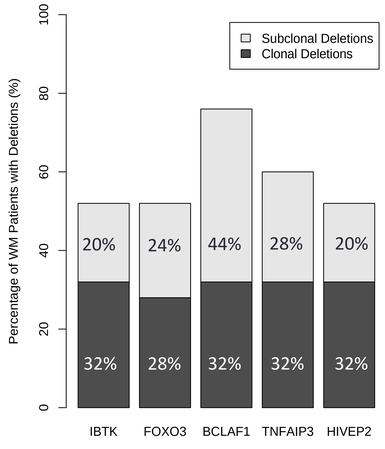
Gender, n (%)		**MYD88 L265P**, n (%)	
Male	18 (72%)	Mutated	25 (100%)
Female	7 (28%)	Wild-type	0 (0%)
Age years, median (range)	62 (35-91)	**CXCR4 WHIM**, n (%)	
% BM Involvement, median (range)	72% (20%-90%)	Mutated	11 (44%)
Serum IgM Levels mg/dl, median (range)	3510 (598-6910)	Wild-type	14 (56%)



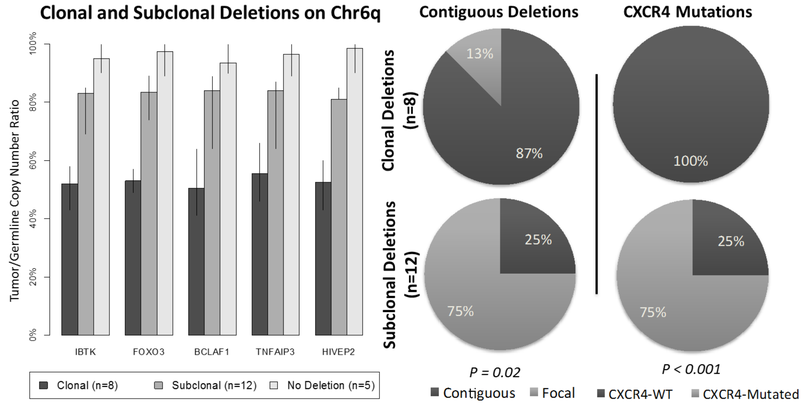
Based on location, function, and existing literature, we investigated the role of IBTK, FOXO3, BCLAF1, TNFAIP3 and HIVEP2 genes as potential targets of chr. 6q deletions. The tumor/germline (T/G) copy number ratio was used to estimate the clonality of observed deletions. Monoallelic deletions affecting less than 20% of cells were considered to be beneath the threshold of detections for the PCR assays.

Conflict of interest disclosures: There are no relevant conflicts of interest to disclose.

RESULTS: CNA PATTERNS CORRESPOND TO CXCR4 MUTATION STATUS



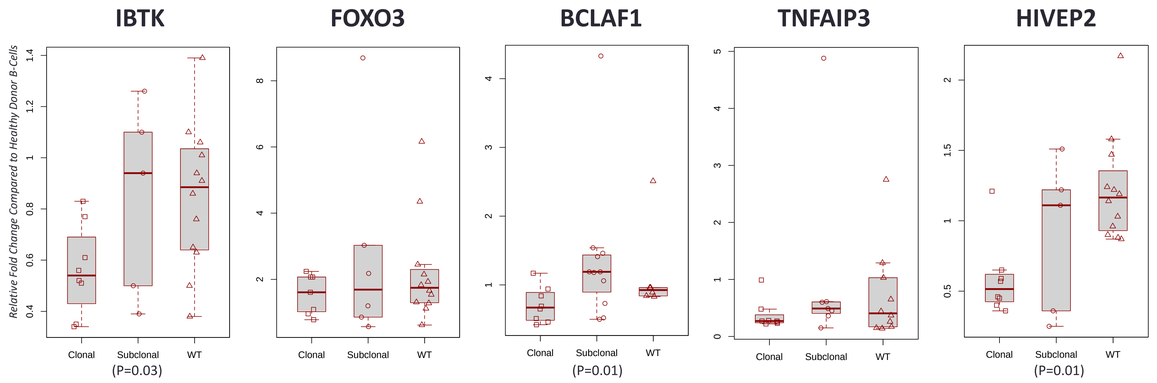
Percentage of WM patients with fully clonal or subclonal chr. 6q deletions by gene.



The median tumor/germline ratio of the subclonal deletions was 0.83 (range 0.64-0.89) corresponding to a monoallelic deletion affecting 34% of LPL cells. Clonality of the deletions was strongly associated with CXCR4 mutations status.

CNA assays revealed absence of copy number changes in both B and non B-cell compartment for all the healthy donors. Somatically acquired fully clonal deletions in chr. 6q were observed in 8/25 (32%) patients. The deletions spanned all studied genes in 7/8 (87%) patients; in one patient, FOXO3 remained fully intact. Subclonal deletions in chr. 6q were observed in 12/25 (48%) patients and, unlike the fully clonal chr. 6q deletions, spanned all 5 studied genes only in 3/12 (25%) cases (p=0.02). Five out of 25 WM patients (20%) presented without any deletion in the studied chr. 6q regions.

RESULTS: GENE EXPRESSION



Gene expression assays for all 25 patients showed significant decreases in the transcriptional levels of IBTK, BCLAF1 and HIVEP2 corresponding to the clonal deletions observed for their respective gene (p= 0.03, 0.01 and 0.01, respectively).

In contrast, gene expression levels for TNFAIP3 and FOXO3 were not affected by gene deletions.

TABLE 2: TARGET GENE FUNCTION

GENE NAME	LOCATION	PROTEIN FUNCTIONS
IBTK	Chr. 6q14.1	1) Direct binding and inhibition of **BTK** activation on **BCR** signaling; 2) PKC-mediated IBTK S87/90-phosphorylation releases active **BTK** from the BTK-IBTK complex resulting in downstream activation of the **NFkB** pathway.
FOXO3	Chr. 6q21	1) Involved in DNA damage-induced **apoptosis** ATM, Chk2 and the phospho-p53 isoform.
BCLAF1	Chr. 6q23	1) Transcriptional repressor that interacts with antiapoptotic **BCL2** protein family members; 2) Transcriptional and translational regulation of different genes involved in DNA damage signaling and repair in the context of the BRCA1/BCLAF1 mRNA splicing complex; 3) Germline variants (SNPs, del, ins) have been associated with increased risk of NHL.
TNFAIP3/A20	Chr. 6q23	1) Negative regulator of the **NFkB** activation induced by TNF or **TLR**; 2) TNFAIP3 inactivation by deletions and/or mutations is involved in B- and T-cell lymphomagenesis and in the context of autoimmunity; 3) Germline abnormalities (SNPs, del, ins) have been associated with increased risk of NHL.
HIVEP2	Chr. 6q23-q24	1) Several regulatory functions, including the transcriptional repression of c-Myc and genes involved in **NFkB** signaling.

TNF: tumor necrosis factor; TLR: Toll-like receptor; SNPs: single-nucleotide polymorphisms.

CONCLUSIONS

- Gene loss of IBTK, FOXO3, BCLAF1, TNFAIP3 and/or HIVEP2 occurs in most patients with WM;
- Gene loss patterns differ between patients with fully clonal deleted chr. 6q and those with subclonal chr. 6q deletions;
- Despite clonal genomic losses in all 5 genes, the expression levels were significantly reduced only for IBTK, BCLAF1, and HIVEP2, suggesting the possibility of their haploinsufficiency in WM while regulatory mechanisms may compensate for FOXO3 and TNFAIP3 gene loss;
- CXCR4 mutations were absent in chr. 6q fully clonal deleted patients, but common in those with subclonal chr. 6q or no chr. 6q deletions;
- The present findings provide valuable insights into WM pathogenesis, and may be relevant to understanding therapeutic outcome with agents that target MYD88, CXCR4, BCL2 and BTK.