Are losses in 6q important to WM growth and survival?

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Deletions involving the long arm of chromosome 6 (del6q) have been observed in about 50% of Waldenström's Macroglobulinemia (WM) patients. Chr6q genomic loss in WM affect important modulators of NF-kB (TNFAIP3, HIVEP2), BCL2 protein family (BCLAF1), apoptosis (FOXO3), and BTK (IBTK). We therefore sought to delineate the gene losses related to del6q in asymptomatic and symptomatic WM and their association to MYD88 and CXCR4 mutations and signaling.

We studied 33 untreated WM (14 $MYD88^{MUT}CXCR4^{WT}$, 11 $MYD88^{MUT}CXCR4^{MUT}$, 8 $MYD88^{WT}CXCR4^{WT}$). All 8 asymptomatic WM were $MYD88^{MUT}$, 5 of whom were also $CXCR4^{MUT}$. The 25 symptomatic WM included 17 $MYD88^{MUT}$, 6 of whom were also $CXCR4^{MUT}$, and all 8 $MYD88^{WT}CXCR4^{WT}$ patients. For the 5 studied genes, copy number alterations (CNA) and gene expression were measured from CD19+ BM lymphoplasmacytic cells/CD19- peripheral-blood mononuclear cells (PBMC) from patients and paired CD19+/CD19- PBMC from 6 healthy donors with TaqMan RT-PCR protocols. Deletions affecting <20% of WM cells were considered below the RT-PCR detection threshold.

CNA assays revealed heterozygous somatic deletions for at least one 6q MDR gene in 20/25 (80%) $MYD88^{MUT}$ WM patients. In contrast, no CNA were present in healthy donors. Deletions for at least one 6q MDR gene were detected in 7/8 (88%) asymptomatic compared to 13/17 (76%; p=NS) symptomatic $MYD88^{MUT}$ patients. Eight $MYD88^{MUT}$Del6q WM (40%) showed more clonal and contiguous losses spanning across all MDR genes, while the remaining 12 (60%)

had more focal and subclonal gene losses. Clonal contiguous deletions were mutually exclusive of CXCR4 mutations. Clonal deletions of IBTK, BCLAF1 and HIVEP2 significantly reduced the corresponding gene transcriptional levels in the 8 clonally 6qdel *vs* all the other $MYD88^{MUT}$ patients. Among all 8 $MYD88^{WT}CXCR4^{WT}$ patients, IBTK remained intact and no contiguous del6q were observed. Moreover, we analyzed previously published RNASeq data and identified 19 genes co-regulated by 6qdel and CXCR4 mutation status in $MYD88^{MUT}$ WM, potentially involved in WM clonal evolution.

Our findings provide new insights into WM pathogenesis, including loss of key regulators of BTK, apoptosis, BCL2 and NF-kB signaling in asymptomatic and symptomatic WM patients, and shared regulatory signaling for $MYD88^{MUT}$ patients with either 6qdel or $CXCR4^{MUT}$ disease.