Chromosome and Gene Expression in Waldenstrom's Macroglobulinemia.

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Over the last decade there has been extensive work performed towards the identification of a genetic basis for WM. Family studies have shown that up to 20% of WM patients may have a familial predisposition, suggesting a genetic predisposition for WM. Moreover, there appears to be an increased incidence of WM among individuals of Ashkenazi decent. Several studies have reported on cytogenetic findings in WM and have demonstrated a great variety of chromosomal abnormalities. Numerical losses involving chromosomes 17, 18, 19, 20, 21, 22, X, and Y have been commonly observed, though gains in chromosomes 3, 4, and 12 have also been reported. The most consistent chromosomal abnormality has been the loss of chromosome 6q deletions in up to half of WM patients encompassing 6q21-22. Recent studies by Chang et al, have extended the region of this deletion to 6q21-25. The frequency of 6q21-22 deletions appears at a comparable frequency amongst patients with and without a familial history. The presence of 6q deletions has also been suggested in one study to distinguish patients with WM versus those with IgM monoclonal gammopathy of unknown significance (MGUS), and to have potential prognostic significance, though Chang et al have found no prognostic significance to the presence of 6q deletions in WM. While 6q deletions have been reported in other B-cell malignancies, and therefore are not WM specific, several candidate "cancer suppressor" genes in this region are under investigation including ***BLIMP-1***, a master regulatory gene implicated in the differentiation of B-cells to plasma cells. Since disruption of ***BLIMP-1*** and genes controlled by it can potentially result in the freezing of B-cell differentiation, thereby resulting in a "WM like" lymphoplasmacytic cell, Leleu et al examined the ***BLIMP-1*** signaling pathway in WM patient tumor cells. These studies showed that the ***BLIMP-1*** pathway did not harbor any mutations. However, variations in two genes in this pathway were seen: ***IRE1*** and ***XBP1***, with higher levels of a spliced form of their transcripts observed in WM patients compared to healthy individuals. Though no mutations in either ***XBP1*** or ***IRE1*** were found to account for these events, a higher incidence of natural variations in DNA structure (polymorphisms) was seen among WM patients in ***IRE1***. While routine bone marrow cytogenetic testing is not recommended as a standard test in WM patients at this time, it may be useful in certain cases where IgM myeloma may be suspected wherein translocations in chromosome 14q32 are a predominant feature.

Since WM represents a disorder with a high familial and Ashkenazi predilection, we recently performed extensive sequencing studies of the ***p53***, ***BRCA1*** and ***BRCA1*** genes including all promoter regions, exons and flanking introns in WM patients with either a Li Fraumeni like family cancer history i.e. family history of WM, breast and GI cancers (for ***p53***) or multiple cancers with an Ashkenazi ethnic background (for ***BRCA1, BRCA2***). No mutations in these genes were identified.

To gain insight into the molecular basis of WM, several investigators have performed gene expression profiling (GEP) studies of WM cells, and surrounding microenvironmental cells. These studies have showed distinct gene expression patterns for malignant and microenvironmental cells in WM patients compared to healthy individuals, including genes involved in proliferation, apoptosis, transcription, as well as in protein trafficking and localization. Hatjiharissi et al showed that WM cells over-expressed genes with a potential role for therapeutic targeting (e.g. ***BCL-2, CD40, TACI, FLIP, cIAP-2, XIAP***) in WM cells. Importantly, this study described a unique molecular profile for microenvironmental cells in WM patients including genes that mediate immune and inflammatory responses (i.e. ***TLR 4,5,7,8, IL-6R, IL-10R, IL-8R***), and genes encoding extracellular matrix components (i.e. ***FN1*** and ***HGF***).

Since most patients with WM demonstrate decreased levels of IgA and IgG, a finding suggestive of a defect in the ability of WM patients to potentially undergo a process known as antibody "class switching", whereby IgM molecules undergo forced mutation to become IgA or IgG molecules, we recently performed extensive sequence analysis of 19 untreated WM patients who demonstrated IgA and/or IgG deficiency (hypogammaglobulinemia) for 8 genes (***AICDA***; ***BTK; CD40; CD154; NEMO***, ***TACI***, ***SH2D1A,*** and ***UNG***) most often implicated in disorders that can mimic the antibody profile found in WM patients; i.e. high IgM levels, along with low IgA and IgG levels. These disorders include common variable immunodeficiency disorders (CVID), hyper IgM syndrome (HIGM), and X-linked agammaglobulinemia (XLA). The most notable finding in these studies was a missense mutation resulting in a highly conserved catalytic domain for the enzyme produced by the ***UNG*** gene: uracil-DNA glycosylase. Uracil-DNA glycosylase is essential to antibody "class switching". Individuals deficient in uracil-DNA glycosylase exhibit a hyper-IgM syndrome, with increased IgM levels and decreased IgA and IgG levels. Moreover, mice deficient in ***UNG*** develop B-cell lymphomas late in life suggesting a tumor suppressor role for uracil-DNA glycosylase. Further validation of these findings, as well as investigation of ***UNG*** and other uracil DNA glycosylases in the pathogenesis of WM are currently underway.

Lastly, the genetic background of patients may also be important for predicting therapeutic response to drug therapies, including rituximab. A correlation has recently been established between certain natural variations in DNA (polymorphisms) which are found in the ***FcγRIIIa*** gene receptor (CD16), a receptor found on immune cells that plays a role in rituximab mediated killing of tumor cells. Individuals may encode either the amino acid valine (V) or phenylalanine (F) at position 158 in this receptor. In studies by Treon et al, WM patients who carried at least one valine amino acid had a fourfold higher chance of attaining a major response (i.e. 50% decline in serum IgM levels) to rituximab versus those patients who carried only phenylalanine. The FDA recently cleared testing for this polymorphism in patients with indolent non-Hodgkin's lymphoma, including WM for assessing likelihood of response to single agent rituximab (see **www.pgxhealth.com**).