[ABSTRACT WM1.4]

INHERITED AND ACQUIRED MUTATIONS IN WALDENSTROM'S MACROGLOBULINEMIA(WM): WM AND MM HAVE CLOSE GENETIC RELATIONSHIPS NOT SHARED WITH B-CLL

L.M. Pilarski,1 S. Adamia,1 A. Reichert,1 A. Ghosh,1 M.J. Mant,2 T. Reiman,1 S.P. Treon,3 A.R. Belch1

1Department Of Oncology, Canada; 2Department of Medicine, University of Alberta and Cross Cancer Institute, Edmonton AB, Canada; 3Dana Farber Cancer Institute, Boston MA, USA

Hyaluronan synthase 1 (HAS1) synthesizes, a highly polymeric sugar molecule that is important in cell motility, signaling and mitosis.1We identified three aberrant splice variants of HAS1 transcripts, two of which are the result of partial intron retention (intronic splicing), that were found only in patients with WM or multiple myeloma (MM).2,3 Intronic splicing has been reported only in cancer cells, and is absent from cells of healthy individuals. For 146 MM patients, expression of the intronic HAS1Vb splice variant was strongly correlated with reduced survival (p=0.005)3 (unpublished). HAS1Vb results from *splicing out* of exon 4 and retention of a segment of intron 4, thereby causing an inframe shift and a new stop codon in exon 5, leading to a truncated protein. HAS1Vc also results from aberrant splicing causing an inframe shift and a new stop codon; HAS1Va which results from deletion of exon 4, an inframe shift and a new stop codon also correlates with reduced survival though at a lower level of significance than is seen for HAS1Vb. Our evidence supports the idea that the truncated HAS1 splice variants are translated and functional in WM and MM. Although the mechanism whereby aberrant HAS1 splicing influences survival is as yet unclear, our analysis of HAS1Vb transfectants shows that HAS1Vb has an intracellular localization to cytoskeletal elements. Although most HA is extra-cellular, HAS1Vb appears to be the only member of the HAS1 family that synthesizes intracellular HA,3 which may alter mitosis.4 Aberrant splicing arises from either (a) sequence variations of the DNA localized in splicing elements, or (b) abnormalities in the splicing factors whose assembly is directed by the specific sequences of DNA i.e. splicing elements, to carry out alternative splicing of nuclear pre-mRNA. Based on the classical dogma of splicing, we predicted that mutational events in splicing regions of the HAS1 gene itself were responsible for altered spliceosome assembly, with consequent aberrant splicing of HAS1 pre-mRNA and significantly reduced patient survival. To determine whether inherited germline origin polymorphisms and/or genetic variations, or acquired HAS1 mutations had the potential to alter premRNA splicing of HAS1 to generate HAS1Vb and the associated reduction in patient survival, we sequenced the regions of genomic HAS1 predicted to control generation of the observed splice variants. Because aberrant splicing was occurring in the region of HAS1 exon 3 to exon 5, we carried out extensive sequencing of this region of genomic HAS1 from 7 MM, 6 WM, 5 B-CLL, 3 MGUS and two healthy donors. To gain an appreciation of the mutation pattern of genomic HAS1, we sequenced exon 3 to exon 5 from buccal cells as well as from sorted subpopulations of hematopoietic cells, including malignant B and plasma cells and nonmalignant T cells. For some patients, we also sequenced HAS1 from sorted CD34+ hematopoietic

progenitor cells. We identified a series of 182 recurrent and unique mutations in HAS1, some of which were germline in origin and hence inherited, and others which were acquired in somatic cells (defined as being present in hematopoietic and/or malignant cells, but absent from buccal cells). Somatic mutations included those found in all populations of hematopioietic cells tested (including CD34+ progenitor fractions, but not in buccal cells) and tumor-specific mutations found only in malignant B and plasma cells. Minor alleles for some inherited HAS1 single nucleotide polymorphisms reported by NCBI were found to be significantly over represented in WM and MM, as compared to healthy donors5,6 (unpublished)(Adamia *et al.* this volume). Unexpectedly, we identified numerous recurrent somatic and germline origin mutations in the sequenced *HAS1* exons and introns - that is the same somatic mutations were found in many to most of the patients analyzed, even though all were unrelated and their cancers were not familial in nature. These were not found in *HAS1* of B-CLL, MGUS or healthy donors analyzed so far. A substantial number of recurrent *HAS1* mutations were shared among MM and WM patients. Most of the recurrent somatic mutations are non-randomly clustered in the vicinity of predicted splicing elements on *HAS1* exons and introns. Furthermore, analyzing the impact of these recurrent mutations on spliceosome assembly using bioinformatic tools predicted a profound impact of mutational clusters on splicing patterns. Provocatively, one cluster of recurrent mutations precisely predicted the splicing pattern required to generate HAS1Vb. We speculate that inherited *HAS1* polymorphisms predispose to WM and MM, but not to B-CLL, followed by progressive acquisition of recurrent *HAS1* mutations in hematopoietic progenitors which pass these mutations to all cells of the hematopoietic lineage as normal differentiation proceeds. These somatic mutations increase the potential for aberrant splicing and hence the risk of developing the disease. Finally acquisition of somatic *HAS1* mutations, both recurrent and unique, occurs in the tumor progenitors, providing a marker for the earliest stages of disease. In combination with the accumulated preceding mutations, cancer -specific mutations, may be the final stage in promoting aberrant HAS1 splicing, thus setting in motion abnormalities that culminate in overt malignancy. The presence of the same somatic mutations independently arising in a group of unrelated patients having either of two otherwise unrelated diseases implies the influence of a strong and consistent selection during oncogenesis for specific *HAS1* mutations during the originating events leading to MM and WM. The presence of shared somatic mutations among MM and WM patients implies that the common early stages of oncogenesis are shared between these two cancers. The cell type in which the culminating genetic events occur determines whether an individual develops MM or WM. Thus, WM and MM have a shared genetic history. In contrast, none of the B-CLL patients analyzed to date have *HAS1* mutations, indicating that WM and B-CLL are unrelated at the early genetic level, despite work showing them to have similar gene expression profiles.7 It seems likely that for WM and B-CLL, shared gene expression profiles reflect common B lineage differentiation stages rather than mechanistically related transformation events or disease-related characteristics. We have found that inherited predispositions and progressively acquired somatic mutations in the *HAS1* gene of individuals at risk of WM and MM correlate with abnormal intronic splicing of *HAS1*, which in turn has a strong correlation with reduced survival. Our preliminary work suggests that this progressive accumulation of recurrent somatic mutations in the hematopoietic lineage and then in the tumor cells themselves

accompanies development of WM and MM, but does not characterize B-CLL. This suggests that *HAS1* may play a central role in a shared oncogenic process contributing to transforming events that underlie both MM and WM. Furthermore, clinical monitoring of patients to determine the mutational status of their HAS1 genes may provide a powerful predictive test for assessing the risk of transformation to overt malignancy. This approach has considerable potential to predict risk for individuals with pre-malignant conditions as well as for monitoring the transition from premalignant to malignant disease. It holds considerable promise for the development of risk assessment strategies, for early detection and monitoring of malignancy before, during and after therapy, and to assess response and predict relapse. As well, the pattern of tumor-specific recurrent somatic mutations is likely to provide a common clonal marker for all such patients. This may enable regular monitoring strategies for every patient to unequivocally identify clinically cryptic tumor cells in the early stages of emerging malignancy, as well as precise molecular monitoring of the response to treatment and stratification of monoclonal gammopathies with the greatest risk of transformation to WM.

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