Genetic Abnormalities in Waldenström's Macroglobulinemia

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Abstract

The genetic factors that lead to WM are mostly unknown but are likely to involve inherited polymorphisms that might be markers of increased risk for developing WM, and somatic mutations that might be acquired during the events leading to oncogenesis and cancer progression. By intensive sequencing of the hyaluronan synthase 1 (HAS1) gene in malignant and normal cells from patients with WM, we have identified both types of mutation in *HAS1* exons and introns. Acquired *HAS1* mutations are found in malignant cells as well as presumptively nonmalignant CD34+ progenitor cells. This suggests that acquired *HAS1* mutations precede frank malignancy and might contribute to the initial transforming events in WM as well as to disease progression.

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Introduction

Inherited polymorphisms appear to be predisposing biomarkers that predict risk of developing cancer. Somatic mutations which influence gene splicing can be transforming or effect disease progression. Genetic analysis can either identify associations by large scale screening followed by functional validation, or can focus on a single gene already known to be clinically important. Recently, whole-genome sequencing in acute myeloid leukemia has identified somatic mutations in a set of 10 mutated genes, 8 of which were previously unlinked to the cancer; these were present in nearly all tumor cells.[1] Using the alternate but related approach for a single gene, hyaluronan synthase (HAS1) known to be important, we found hypermutation in exons and introns on this gene at a frequency of about 0.5pb/100bp.[2] This study included 17 patients and 23 control subjects. Previously, we have shown that the *HAS1* gene is aberrantly spliced in malignant cells and that in a cohort of patients with multiple myeloma (MM), aberrant splicing of this gene predicts significantly reduced survival.[3] Moreover, one variant of HAS1, HAS1Vc, is transforming in vitro and in vivo (submitted). In Waldenström's macroglobulinemia (WM) and MM patients we have identified 3 splice variants of HAS1, HAS1Va, HAS1Vb, and HAS1Vc.

In WM, we have identified hypermutation leading to numerous *HAS1* genetic changes, including inherited polymorphisms, acquired somatic mutations, and tumor-specific mutations.[2] We have confirmed that these *HAS1* mutations can lead to clinically relevant aberrant splicing.[2] The *HAS1* mutations we identified in patients appear to be extremely rare or absent in the general population. Of particular interest, they are found in otherwise apparently normal hematopoietic progenitor cells, suggesting that *HAS1* genetic variations are very early genetic changes in the transformation to WM. Our study suggests that those who develop *HAS1* mutations appear to have a high probability of developing cancer, based on the frequency of some *HAS1* genetic variations in WM and MM patients but their absence from B-cell chronic lymphocytic leukemia (B-CLL) and healthy donors. The *HAS1* genetic variations appear to be absent from most monoclonal gammopathies of undetermined significance (MGUS), a frequent condition that evolves to overt WM or MM. *HAS1* genetic variations might identify the subset of MGUS most at risk for transformation to overt disease. All WM patients whose DNA has been sequenced have hypermutated HAS1, with 3-22 mutations per patient. Waldenström macroglobulinemia and MM patients appear to harbor a cascade of somatic hypermutations, starting in apparently healthy hematopoietic progenitor cells and accumulating in cancer cells (Figure 1).[2] *HAS1* mutations may also characterize solid cancers, some of which have already been reported to overexpress *HAS1*.

Aberrant Splicing of Hyaluronan Synthase 1 in Waldenström's Macroglobulinemia

Aberrant splice variants of HAS1 are expressed in WM (and MM), but absent from healthy cells.[3-5] Analysis of hyaluronan (HA) synthesis by MM B cells[3] and transfectants (submitted), suggests that HAS1Va synthesizes extracellular HA, perhaps involved in motility and cancer spread, while HAS1Vb synthesizes intracellular HA, likely involved in mitotic events. In MM, aberrant HAS1 intronic splicing occurs only in circulating B cells and its expression at the time of diagnosis is a strong indicator of poor survival. Recently, HAS1Va has been reported in bladder cancer.[6] In NIH3T3 transfectants, HAS1Vc confers anchorage independence and HAS1Vc-transfected NIH3T3 are tumorigenic in Balb/c mice (Ghosh et al, submitted).

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Our working hypothesis is that inherited predispositions and hypermutation in the *HAS1* gene promote aberrant splicing, which in turn leads to the production of intronic HAS1 splice variants, intracellular synthesis of HA by these splice variants, and alteration of RHAMM localization patterns inside the cell, with consequent effect on mitosis in WM cells. At the intracellular level, the effect of HAS1 could be manifested as increased genetic abnormalities with direct oncogenic potential and/or by predisposing cells to additional oncogenic changes that might enable clonal survival and expansion of malignant cells. Because HAS1 is not expressed by normal blood and bone marrow cells,[3] therapeutic ablation of *HAS1* gene expression might selectively attack the WM clone.

Genetic Variations in Genomic DNA Might Promote Splicing Within HAS1[2,7]

We have sequenced exons 3, 4, 5 and introns 3 and 4 of the *HAS1* gene from 7 WM and 10 MM patients as well as 11 MGUS, 4 B-CLL and 8 healthy donors. In WM and MM we identified 197 genetic variations/single nucleotide polymorphisms (SNPs)[2] (Table 1). The 188 novel genetic variations identified by us, together with 11 previously reported NCBI SNPs, were mapped with the sequences of splicing elements (5' splice site, 3' splice site, branch points and polypyrimidine tracts, exonic and intronic *cis*-splicing elements, splicing enhancers and suppressors) that are necessary for correct splice-site identification and spliceosomal assembly. The 124 inherited and the 75 somatic *HAS1* genetic variations found in WM and MM were frequently located in splicing elements. The novel genetic variations were not detected in a control group of 23 individuals. Inherited genetic variations were present in all cell subsets from a given patient. Somatic genetic variations were found (1) in all hematopoietic subsets sequenced (termed hematopoietic origin), or (2) only in malignant B and plasma cells (PC; termed tumor specific; see below).

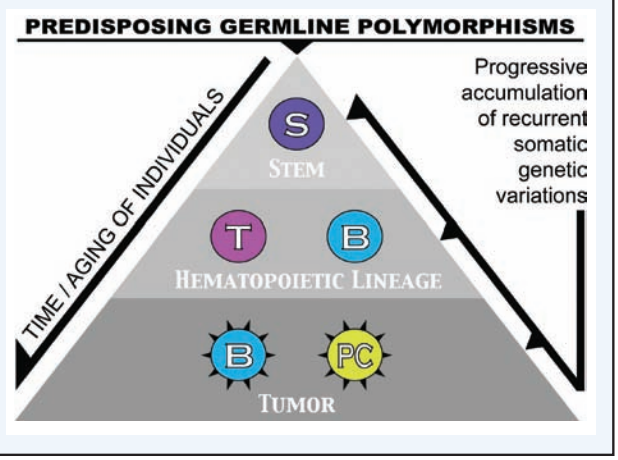
The term "recurrent" is used to designate genetic changes that recur in more than 1 individual (Table 1). We detected 48 recurrent genetic variations in HAS1 which were associated with WM and/or MM, with 19 (40%) recurring in both WM and MM, including somatically acquired genetic variations. The recurrent set included 48 HAS1 genetic variations comprised of 31 inherited genetic variations, 11 acquired hematopoietic lineage genetic variations and 7 acquired tumor-specific genetic variations. None of the somatic genetic variations and only 4 of the germline genetic variations were detected in 23 control subjects.[2] The processes leading to HAS1 hypermutation are as yet unknown but might involve some form of antigen-independent somatic hypermutation.[8] Provocatively, expression of IgH and IgK immunoglobulin genes occurs in hematopoietic stem cells from aged mice but not from young mice.[9] This suggests that enzymes associated with somatic hypermutation might be active in stem cells from aged humans, providing a potential mechanism for early acquisition of somatic mutations in WM hematopoietic progenitor cells (HPC).

Sequential Acquisition of *HAS1* Genetic Variations in WM Patients (Figure 1)

Novel Inherited Genetic Variations

These genetic variations are inherited through the germline and are detected in all cells of a given individual (ie, in buccal cells, B, T, PC, and CD34+ HPCs). These germline genetic variations appear to predispose individuals to cancer as indicated by their frequent presence in WM patients.

Figure 1 Sequential Accumulation of *HAS1* Genetic Variations in Patients with WM and MM



This diagram illustrates the postulated inheritance of predisposing germline mutations in *HAS1* that increase the risk for developing WM, followed by accumulation of somatic mutations in the hematopoietic lineage, starting with HPC (stem cells; labeled "S"). These same somatic mutations are passed to the normal T and both normal and malignant B-cell progeny of HPC, followed by further accumulation of tumor-specific somatic mutations in malignant B and PC (spiked cells, bottom layer).

Table 1 Recurrent *HAS1* Mutations in WM and MM Patients and Control Subjects

Sample Type	Total Number of Genetic Variations* for MM/WM	Recurring Inherited Genetic Variations	Recurring Somatic Genetic Variations	
			Hematopoietic Origin	Tumor Origin
WM	109/197	27/31	6/11	8/8
MM	117/197	28/31	9/11	3/8
IgM MGUS	11/197	10/31	1/11	0
B-CLL	7/197	7/31	0	0
Healthy Donors	11/197	11/31	0	0

*A total of 197 *HAS1* genetic variations were detected in WM and MM. Most of these were unique to only one patient. Only the 48 recurrent genetic variations (those detected in > 1 patient at 48 chromosomal locations in *HAS1*: one position included 3 allelic variants) are listed in columns 3-5. Recurring inherited genetic variations (column 2) include known NCBI SNPs and novel germline *HAS1* genetic variations detected by us.
Abbreviations: B-CLL = B-cell lymphocytic leukemia; MGUS = monoclonal gammopathy of undetermined significance; MM = multiple myeloma; WM = Waldenström's macroglobulinemia

Hematopoietic Involvement

Genetic variations that are specific to the hematopoietic linage are detected in HPC and T cells (ie, healthy hematopoietic cells of the patient). These genetic variations are also found in malignant B lineage cells whose parent B cell arose from HPC, as occurs in WM. They are not germline genetic variations and thus not heritable, as defined by their absence from a representative nonhematopoietic healthy tissue, in this case buccal cells. They are absent from B-CLL, MGUS, and healthy donor hematopoietic cells whose gene segments

we have sequenced. Genetic variations specific to hematopoietic cells might predict an advanced predisposition to malignant disease or clinically cryptic but emerging disease. In HPCs from WM and MM, somatic *HAS1* genetic variations are detected, as defined by their absence from buccal cells. The presence of *HAS1* genetic variations might confer some type of growth advantage because, by definition, at birth all HPCs must have had a HAS1 genotype identical to that of buccal cells. The frequency of these genetic variations in HPC from an adult WM or MM patient is often relatively high (30%-50% of subclones) suggesting that mutated HPCs have become a dominant population. Nevertheless, these are apparently normal HPCs based on their ability to give rise to T cells which are not part of the WM or MM clone but which do harbor the somatic *HAS1* genetic variations passed down from the HPC. These HPCs also pass somatic *HAS1* genetic variations to their B-cell progeny, including malignant B lineage cells. Independent acquisition of the somatic *HAS1* genetic variations in multiple unrelated individuals with MM or WM, suggests that the recurrent somatic *HAS1* genetic variations might increase the probability of transformation to overt malignancy.

Tumor Specific

Tumor-specific somatic *HAS1* genetic variations occur in malignant B lineage cells of WM patients, some of which are shared in B and PC of MM patients. The presence in PC of somatic mutations acquired in WM or MM B cells provides support for the idea that malignant PC are generated from malignant B-cell precursors. Acquired tumor-specific genetic variations, some of which are recurrent, might define clinically cryptic disease. The recurrent tumor-specific genetic variations provide a HAS1 molecular "signature" for use in diagnosis and/or monitoring of response to therapy and disease progression.

Our working hypothesis predicts that, in WM, genetic variation in the *HAS1* gene promotes alternative HAS1 splicing, leading to intronic HAS1 splice variants, and intracellular synthesis of HA by HAS1 variants. It remains to be determined whether somatic mutations can promote aberrant splicing independent of inherited mutations in *HAS1*, or alternatively if both types of mutation are required to promote this process. HAS1 and its variants may synergize with other oncogenes to promote the emergence of increasingly aggressive disease in WM, perhaps by altering mitosis and/or by promoting chromosomal missegregation. Overexpression of *HAS1* and/or *HAS1* variants might help to determine the ultimate balance between apoptosis/death or viability and clonal emergence. HAS1 thus represents a new type of marker that reflects biologically important properties of a malignant clone as it undergoes stepwise oncogenesis and/or disease progression. Because the HAS1 variants appear to be absent from healthy cells, they might present valuable clinical targets for development of new therapeutics that are highly selective for malignant cells.

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