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The significance of spliceosome mutations in chronic lymphocytic leukemia

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Abstract

Cellular proteins produced *via* alternative splicing, provide neoplastic cells with survival advantage and/or promote neoplastic cell proliferation. Pre-mRNA is spliced by the spliceosome consisting of large complexes of small nuclear RNA (snRNA) and protein subunits. Spliceosome gene mutations were detected in 40 – 80% of patients with myelodysplastic syndrome (MDS), particularly in those with ringed sideroblasts. Recently, two large whole genome sequencing studies identified mutations in the spliceosome gene *SF3B1* in approximately 10% of patients with chronic lymphocytic leukemia (CLL). Intrigued by these findings, we performed a pathway enrichment analysis and found that unlike in MDS, in CLL spliceosome mutations exist almost exclusively in *SF3B1*. CLL patients with an *SF3B1* gene mutation are characterized by a short progression-free survival and a low 10 year-survival rate. Furthermore, the frequency of *SF3B1* mutations is significantly higher in chemotherapy treated than in untreated patients with CLL, suggesting that chemotherapy induces *SF3B1* gene mutations or selects a population of mutated cells. Whether *SF3B1* gene mutations have a role in leukemogenesis, either because of altered splicing or other splicing-unrelated functions such as ectopic expression of *Homobox* (*Hox*) genes previously reported in *SF3B1*$^{+/-}$ mice, remains to be determined.

Keywords

CLL; spliceosome mutations; secondary AML; MDS

Introduction

The spliceosome is a large complex of small nuclear RNA (snRNA) and protein subunits that in tandem remove introns from transcribed pre-mRNAs (also termed hnRNA) segments. Spliceosomes are formed for every pre-mRNA in a stepwise process by which discrete snRNA fragments and associated proteins are assembled [1]. The spliceosome plays a major role in gene function, as alternative splicing of a gene alters its post-transcriptional function. For example, alternative splicing of apoptotic pathway genes results in production of proteins with disparate functions, such that one isoform promotes apoptosis while the other isoform of the same gene protects the cell from apoptotic cell death [2]. Cancer cells were shown to “take advantage” of splicing variants and produce proteins that promote neoplastic

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cell growth and survival [3]. Recent whole-genome sequencing analyses identified spliceosome mutations in chronic lymphocytic leukemia (CLL) cells. To further decipher the pathobiological significance of these spliceosome mutations, we re-analyzed published deep sequencing data and reviewed the current literature.

Myeloid Neoplasms

Myeloid neoplasms were the first hematopoietic malignancies shown to have spliceosome gene mutations [4-6]. Spliceosome mutations occur frequently in myelodysplastic syndrome (MDS) and in secondary acute myeloid leukemia (AML) but not in *de-novo* AML or myeloproliferative neoplasms (MPN). Yoshida *et al.*, who conducted a whole-exome sequence analysis of 29 MDS bone marrow (BM) specimens, detected a high rate of RNA splicing machinery mutations [6]. Further analysis of BM samples from 582 patients with a hematologic malignancies including 24 with acute lymphoblastic leukemia (ALL), and 87 with Hodgkin lymphoma (HL) identified spliceosome gene mutations in 84.9% of patients with MDS who had ringed sideroblasts and 43.9% who did not have ringed sideroblasts, 64.5% of patients with chronic myelomonocytic leukemia (CMML), 25.8% of patients with secondary AML, 9.4% of patients with MPN, and a very small number of patient with *de-novo* AML. Spliceosome mutations were not found in ALL or HL. Because these mutations were detected in MDS, CMML, and secondary AML, and because these genes (except for *PRPF40B*, whose function is poorly understood) are known to be engaged in the initial steps of RNA splicing, it was suggested that the identified spliceosome mutations play a role in the pathogenesis of these diseases [4-7].

Because only one mutation was detected in almost all MDS samples harboring a spliceosome mutation, it was assumed that spliceosome mutations are mutually exclusive, and the significance of that “phenomenon” was discussed extensively. According to simple probability rules, it is unlikely that two rare mutations be detected in one sample. The probability of two independent mutations to occur concomitantly is a product of the probability of each mutation to occur separately. For example, mutations in *U2AF35* and *SRSF3A1* were most commonly detected in samples of 155 patients with MDS without ringed sideroblasts. Each mutation was found in 11.6% of the patients. Therefore two mutations were expected to be found in 1.3% ($0.116 \times 0.116 = 0.013$) of the patients, consistent with the data showing that 1 of 155 patients carried both mutations. Therefore, by using this database one cannot determine whether spliceosome mutations in MDS are mutually exclusive.

Chronic Lymphocytic Leukemia

Although no spliceosome gene mutations were detected in ALL or HL, they were detected in CLL. In fact, two large whole-genome sequencing studies identified somatic gene mutations in CLL. Wang and colleagues performed massively paralleled sequencing sequencing in 91 peripheral blood (PB) samples, 61 from patients with untreated CLL and 30 from patients previously treated for CLL. They found a mean of 20 nonsynonymous mutations in each patient [7]. In another exome sequencing study, conducted by Quesada *et al.* in PB samples from 105 untreated patients with CLL, an average of 45 mutations was

identified in each sample [8]. Both studies detected *SF3B1* gene mutations in a significant number of patients. *SF3B1* mutation rate was 15% in the Wang study and 9.7% in the Quesada study.

The high rate of spliceosome gene mutations in MDS led to the assumption that spliceosome mutations play a role in the pathogenesis of this disease. This led us to wonder about the role of spliceosome mutations in CLL. To determine the number and frequency of spliceosome gene mutations in CLL, we performed a pathway enrichment analysis of published CLL sequencing data. First we determined the expected number of mutated genes in this cohort of patients and then compared it to the observed number of mutations within the spliceosome pathway. The expected number of spliceosome gene mutations depends on the proportion of spliceosome genes in the entire genome, the average number of somatic mutations per sample, and the number of samples in the study. According to the KEGG pathway database (http://www.genome.jp/kegg/pathway.html), the spliceosome machinery comprises 120 genes, 115 of which are protein-coding genes. Twelve protein-coding genes directly participate in processing pre-mRNA. Those include *SF3AS1, SF3B1, U2AF35 (aka U2AF1), SRSF2 (aka U2AF2) ZRSR2, PRPF40B, PRPF8, CPSF2, TBM39, COX4*, and *COX3X*. According to the latest estimate, the number of protein-coding genes in the human genome is 20,687 [9]. Hence, spliceosome genes (excluding *SF3B1)* comprise 0.005% (11/20687 × 100) of the human genome. Therefore, the expected number of spliceosome gene mutations (excluding *SF3B1* mutations for which a separate analysis is provided) in the study by Wang and colleagues was 9.1 (0.005% × 20 × 91), and in the study of Quesada *et al.* was 23.25 (0.005% × 45 × 105).

In both studies, various spliceosome gene mutations were detected, albeit at significantly lower frequencies than the *SF3B1* mutation. Wang *et al.* reported single case mutations in 8 spliceosome genes (*RBMX, EIF4A3, PRPF8, USP39, SF3A2, U2AF2 EFTUD2, and DDX46*) whereas Quesada *et al.* found mutations in 9 spliceosome genes in 12 patients (*RBMX, CDC5L, EIF4A3, PRPF19, NCBP2, U2AF2, SFRS7, and MAGOH*). Because the number of spliceosome mutations was lower than expected by our analysis, we concluded that there was no enrichment in spliceosome mutations in either cohort of patients. Hence, in sharp contrast to MDS and secondary AML in which mutations in several spliceosome genes including *SRSF2, ZRSR2, SF3A1, PRPF40B, U2AF65* (also known as *U2AF2*), and *SF1* were identified, in CLL the only gene in which spliceosome mutations were detected in a significant number of patients was *SF3B1*.

The presence of multiple spliceosome gene mutations in MDS and secondary AML suggests that myeloid hematopoietic precursors are susceptible for acquiring spliceosome abnormalities and/or that spliceosome mutations play a pathogenetic role in these hematologic malignancies. In contrast, the CLL sequencing data suggests that *SF3B1* but no other spliceosome gene, plays a role in the pathogenesis of the disease. Mutations in the *SF3B1* gene were found in several hematologic and non-hematologic malignancies, including MDS, AML, CLL, myelofibrosis [4,10], breast cancer [11], skin cancer, and prostate cancers (COSMIC database, http://www.sanger.ac.uk/perl/genetics/CGP/cosmic?action=bycancer&ln=SF3B1), suggesting that *SF3B1* gene mutations have a central role in these tumors' transformation process, either because of the gene's splicing activity or

because of other unidentified splicing-unrelated functions. Indeed, *SF3B1*-null mouse homozygotes died during pre-implantation while, remarkably, no reduction in splicing activity was found in heterozygous *SF3B1*$^{+/-}$ mice despite a 75% reduction in *SF3B1* gene levels [12]. Unexpectedly, ectopic expression of *Hox* genes along with several skeletal abnormalities was noted in these heterozygous mice.

The *Hox* genes are a family of DNA binding transcription factors that were initially identified as regulators of positional identity along the anterior-posterior body axis [13]. Gene expression analysis, gene targeting experiments, and retroviral overexpression studies in a murine BM transplantation model provided strong evidence for the involvement of clustered *Hox* genes in normal hematopoiesis [14]. Furthermore, our group has shown that transfection of mesenchymal cells and dermal fibroblasts with *HOXB2, HOXB4 and HOXB5* converted these cells into hematopoietic cells [15]. The upregulation of *HOX* gene expression in leukemia cells, the leukemogenic fusion of *HOX* with NUP98, and the essential role of *HOX* in the oncogenicity of MLL gene fusions all point to a direct role for *HOX* genes in leukemogenesis [14]. The *SF3B1 gene knockout* data provide sufficient grounds to test whether overexpression of *HOX* genes in *SF3B1*-mutated hematopoietic cells contributes to leukemogenesis. Direct evidence for the role of mutated *SF3B1* in the leukemic transformation process emerged from a recent study showing that *SF3B1* haploinsufficiency results in formation of ringed sideroblasts [16].

The presence of an *SF3B1* mutation correlates with clinical and laboratory parameters. In MDS, patients with an *SF3B1* mutation had a better prognosis. However, because *SF3B1* mutation was found mainly in patients with refractory anemia (RA) with ringed sideroblasts (RS) or refractory cytopenia with multi-lineage dysplasia (RCMD)-RS categories, the prognostic values can be fully accounted for by the World Health Organization (WHO) classification. When RARS and RCMD-RS were analyzed separately, *SF3B1* mutations did not add any prognostic value. [17,18]. In CLL, patients with *SF3B1* mutations had high β2-microglobulin levels, a higher frequency of unmutated *IgHV* gene, an 11q chromosomal abnormality [8,19], a shorter time to disease progression, and a lower 10-year survival rate [8,20].

The *SF3B1* mutation was not detected in patients with monoclonal B-cell lymphocytosis [21]. It was identified in only 5% of 301 untreated CLL patients and in 17% (10 of 59 cases) of previously treated CLL patients, many of whom had fludarabine-refractory disease [20]. Because the *SF3B1* mutation was detected in a significant number of patients with MDS, we wondered whether exposure to chemotherapy leads to acquisition of the *SF3B1* mutation, thereby contributing to the development of secondary MDS in patients with CLL. Overall, of the 196 patients reported in the Wang and Quesada studies, the *SF3B1* mutation was detected in 24 (12.2%), and the mutation rates in untreated patients were similar in both studies (9.5% for Quesada *et al.* and 11.4% for the Wang study). The frequency of the *SF3B1* mutation in 166 patients with untreated CLL was 10.2% (N = 17), whereas it was 23.3% (N = 7) in patients with prior exposure to chemotherapy ($P = 0.044$). Remarkably, prior exposure to chemotherapy was not associated with an increased rate of nonsynonymous mutations in CLL [19].

Taken together, these findings are consistent with the hypothesis that *SF3B1* mutations play a role in the pathobiology of CLL and predispose CLL patients to the development of MDS and possibly other secondary neoplasms. The high *SF3B1* mutation rate in previously treated CLL patients suggests that the *SF3B1* mutation is induced by chemotherapy. Sequential studies in patients with CLL who developed secondary malignancies would likely address this question.

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