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Whole exome sequencing identifies a recurrent *NAB2-STAT6* fusion in solitary fibrous tumors

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Solitary fibrous tumors (SFTs) are rare mesenchymal tumors. Here, we describe the identification of a *NAB2-STAT6* fusion from whole exome sequencing of 17 SFTs. Analysis in 53 tumors confirmed the presence of seven variants of this fusion transcript in 29 tumors (55%), a lower bound for fusion frequency at this locus, suggesting that the *NAB2-STAT6* fusion is a distinct molecular feature of SFTs.

To better understand the molecular lesions contributing to SFT, we performed whole exome sequencing of DNA isolated from SFTs and matched blood from 17 patients (Supplementary Table 1). On average, we sequenced a median of 28.9 Mb per tumor, and 87.5% of the captured exons were covered to a depth of 20× or greater. An average of 22.5 non-synonymous somatic mutations per tumor were observed across the 17 samples

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Contributions: JC and MM conceived the project. JC, AMC, and MM wrote the manuscript with input from all co-authors. JC, AMC, and RO performed experiments. JC and MR performed computational analyses. AM contributed an unpublished algorithm to the analysis. JC, AMC, MR, SRW, RO, DF, and MM analyzed the results. AMC and MCH contributed samples. LA and DA facilitated transfer, sequencing, and analysis of samples.

Conflicts of interest: MM is a paid consultant for and equity holder in Foundation Medicine, a genomics-based oncology diagnostics company, and is a paid consultant for Novartis. MCH is a paid consultant for and equity holder in Molecular MD, a molecular diagnostics company, and is a paid consultant for Novartis.

(median: 19; range: 12-41; median rate of 0.66 mutations/Mb; Supplementary Fig. 1). This corresponded to 390 somatically mutated genes, of which one gene mutated in two samples (*RBPJ*) reached nominal statistical significance for recurrence ($q < 0.1$; Supplementary Tables 2 and 3; Supplementary Methods). Read count analysis of the tumor copy number profiles showed 11 tumors with structurally undisrupted genomes and no obvious gains or losses, while six tumors showed a broad loss of chromosome 13, including two samples with a concurrent broad gain of chromosome 8 (Supplementary Fig. 2).

Given that many soft tissue tumors are driven by genomic translocations,¹ we explored the possibility that gene fusions may be contributing to tumorigenesis in SFT. Using whole exome sequencing data, our fusion detection was limited to breaks that occurred within exons or near intron-exon boundaries. However, we identified 19 potential fusion events in 10/17 tumor samples using algorithms designed for this application (Supplementary Table 4; Supplementary Methods).

Rearrangements in 7/17 tumors represented in-frame fusions of the *NAB2* and *STAT6* genes both located on chromosome 12 (Supplementary Fig. 3a). Sequence review identified paired-end reads where one read mapped to an exonic region of *NAB2* and its pair mate mapped 1.8 to 5.2 kilobases before the transcription start site of *STAT6* (Supplementary Fig. 3b; Supplementary Table 4). Given that sequence mate pairs representing the two ends of a DNA fragment were mapped in opposite orientations on the same strand (Supplementary Fig. 3b), we concluded that this fusion was likely due to an intra-chromosomal inversion that juxtaposed *NAB2* and *STAT6* (Fig. 1a). Normally, *NAB2* and *STAT6* are in opposite orientations but the predicted inversions bring the two genes in close proximity in the same orientation (Fig. 1a). In all cases, we also identified at least one sequence that spanned the *NAB2/STAT6* fusion boundary. We confirmed the genomic breakpoint in two tumors by sequencing of PCR products generated with breakpoint-spanning primers (Supplementary Fig. 4). The fusion was not observed in DNA from matched normal tissue (data not shown).

To analyze the expression and frequency of *NAB2-STAT6* fusions, we performed RT-PCR using primers placed within exons 2-3 of *NAB2* and within exon 1 of *STAT6*, and cDNA generated from 53 tumor samples representing 48 patients (Supplementary Table 1). An RT-PCR product was identified in 27 tumors (51%) from 23 patients. Sequencing of these products revealed multiple isoforms of the fusion that varied in the location of the fusion within *NAB2* (Fig. 1b, Supplementary Fig. 4). The *NAB2* sequence was followed by a sequence belonging to the 5' untranslated region of *STAT6*. Comparing the cDNA structure to the inversion structure identified at the genomic level indicates that the region between exon 5 of *NAB2* and the beginning of *STAT6* (~10kb) is spliced during transcription or that a large portion of this intronic region is deleted when the chromosome is rearranged. Additionally, we identified rare *NAB2-STAT6* fusions that occurred in downstream exons of *STAT6* in 2/17 cases from whole exome sequencing data (see Supplementary Table 4).

NAB2 encodes a transcriptional repressor of the zinc finger transcription factors EGR1 and EGR2, effectors of TGF β signaling in smooth muscle.^{2,3} *NAB2* contains two conserved domains that interact with EGR1 to mediate multimerization and repress transcription, respectively.^{2,3} Functional alterations of *NAB2* have not been demonstrated in cancer but

loss is reported in prostate cancer, lung cancer, non-Hodgkin's lymphoma, and neuroblastoma, and overexpression in melanoma, Ewing sarcoma, and rhabdomyosarcoma.⁴⁻⁶

STAT6 is a transcription factor that modulates signaling by IL-4 and IL-13 in the immune system.⁷ Activation of STAT family members in cancer underlies the hypothesis that these proteins are potential therapeutic targets.⁸ STAT6 in particular has been associated with increased proliferation and invasiveness in glioblastoma.⁹ Based on the properties of NAB2 and the oncogenic potential of STAT6, we hypothesize that the NAB2-STAT6 fusion dimerizes through the oligomerization domain of NAB2, translocates to the nucleus, and modulates STAT6-dependent gene expression. In experimental systems, fusion of STAT3 to a portion of the estrogen receptor results in a similar phenomenon.¹⁰

In summary, we have identified a novel *NAB2-STAT6* fusion in at least half of SFT tumors from whole exome sequencing data. Although most fusions are identified from whole genome or whole transcriptome sequencing, this finding validates the use of exome data for the discovery of fusions that occur mid-exon. The *NAB2-STAT6* fusion appears to be unique to SFT samples as fusion analysis of approximately 713 unique tumor-normal pairs from 5 tumor types analyzed by whole genome, exome, or transcriptome sequencing, or a combination of these techniques,¹¹⁻¹⁵ failed to identify any fusions involving these genes. As such, this fusion represents the first molecular feature unique to SFTs. These data also suggest that small molecule inhibitors of STAT6 may be efficacious in this tumor type. Further experiments investigating the functional behavior of this fusion protein are ongoing. Our estimates of the frequency of *NAB2-STAT6* fusions should be considered a lower bound because whole exome sequencing would fail to identify intronic breaks, and because our RT-PCR approach is limited to the regions covered.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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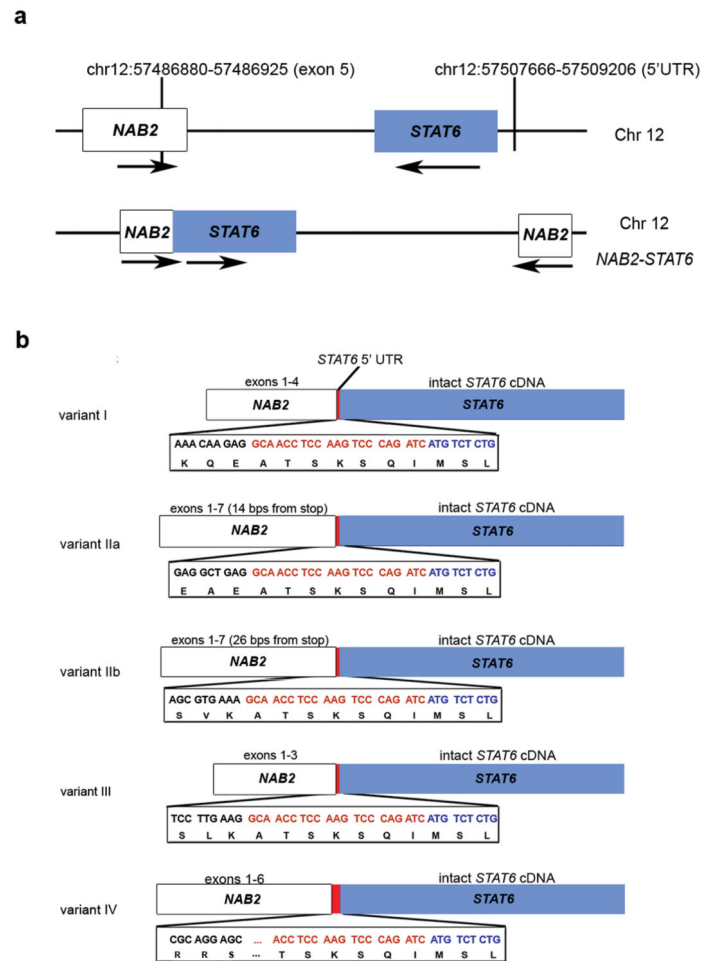


Figure 1. Structure of *NAB2-STAT6*

(a) An inversion within chromosome 12 results in the juxtaposition of the *NAB2* and *STAT6* genes. (b) Schematic diagram of five representative fusion variants showing a conserved breakpoint within *STAT6* and variable lengths of *NAB2*. White regions represent *NAB2*, red regions correspond to the 5' untranslated region of *STAT6*, and blue regions show *STAT6*.