Distinct Clinicopathologic Features Of Nab2-stat6 Fusion Gene Variants In Solitary Fibrous Tumor With Emphasis On The Acquisition Of Highly Malignant Potential.

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Introduction: Solitary fibrous tumors (SFTs) are classified as intermediate (rarely metastasizing). Although the histological criteria of malignant SFT have been proposed, the biological mechanism of SFT remains to be elucidated. Diagnosis of SFTs has classically been based on the immunohistochemical expression of specific markers, such as CD34, but CD34 expression can be typically absent in malignant SFTs and areas of dedifferentiated SFTs. Recently, nuclear STAT6 expression was identified as a highly sensitive and specific immunohistochemical marker for SFTs following the discovery of NAB2-STAT6 as a SFT-specific chimeric fusion gene. The impact of secondary genetic alterations on tumor biology in translocation-associated sarcomas has also been reported, such as p53, PDGFRβ, and telomerase reverse transcriptase (TERT). Recent studies demonstrated that these mutations occur in a subset of SFTs.

In this study, we examined variations of the NAB2-STAT6 fusion gene in 40 cases of SFTs together with the genetic alterations of p53, PDGFRβ and TERT promoter, and compared these genetic features with the characteristic clinicopathologic features.

Methods: Forty-three samples of SFT from 40 patients were collected. Two cases were diagnosed as dedifferentiated SFTs. Histological factors that were evaluated, include, cellularity, mitotic count, nuclear atypia/pleomorphism, necrosis, and hemorrhage. With regard to the DSFT cases, histologic evaluation was performed in both conventional and high-grade areas. Total RNA was extracted from each formalin-fixed paraffin-embedded (FFPE) block and cDNA was synthesized for RT-PCR. A total of 16 types of fusion transcripts were examined by a single PCR. The PCR primers were partly validated by using artificially prepared sequences for each fusion transcript. Genomic DNA was extracted from each FFPE block. Mutations in the p53 (exon5-9), PDGFRβ (exon12, 14, 18-20), and the polymorphisms at the TERT promoter region were examined using PCR. Statistical analysis was performed using the Chi-square test, Mann-Whitney U test and Kaplan-Meier analysis. P-values < 0.05 were considered to be significant.

Results: Among 38 conventional SFTs, 23 cases were from the thoracic cavity (pleura, lung, and diaphragm), 9 were from soft-tissues, and 6 were from the central nervous system (brain and orbita). The 2-year and 5-year disease-free survival rates were 90.6% and 83.1%, respectively. With regard to the two cases of DSFTs, mitotic figures were frequently observed in the dedifferentiated areas of both SFTs, whereas mitosis was not observed in the conventional area of these SFTs. In immunohistochemistry, thirty-eight of 40 samples (95%), including two cases of DSFTs, were positive for CD34 expression. In both cases of DFSTs, CD34 expression was only observed in the conventional areas but lost in the dedifferentiated areas. In contrast, all 40 samples demonstrated strong nuclear
staining for NAB2 and STAT6. Strong nuclear expression of STAT6 was also observed in one CD34-negative case.

In conventional SFT cases, 17 were categorized as p53-positive, and p53-positive cases showed statistically higher Ki-67 LI, higher mitotic rates (mitosis>4/HPF) and the presence of nuclear atypia/pleomorphism compared to p53-negative cases. Moreover, p53-positive staining was associated with a lower disease-free survival rate. In dedifferentiated cases, labeling indices for Ki-67 and p53 were significantly higher in the high-grade areas.

The NAB2-STAT6 fusion gene was detected in all 40 cases, including, those of dedifferentiated SFTs. Among the 16 types of fusion variants screened, 7 types of NAB2-STAT6 fusion variants were observed in this study. NAB2 exon4-STAT6 exon2 appeared most frequently in 18 cases, 16 of which were observed in thoracic cavity. Other fusion variants include, NAB2 exon2-STAT6 exon6 in 7 cases, NAB2 exon6-STAT6 exon18 in 5 cases, NAB2 exon2-STAT6 exon2 in 4 cases, NAB2 exon4-STAT6 exon3 in 3 cases, NAB2 exon6-STAT6 exon16 in 2 cases, and NAB2 exon7-STAT6 exon2 in 1 case.

NAB2-STAT6 fusion variants displayed some distinct clinicopathological features. There were statistically significant correlations between tumors with either NAB2 exon4 -STAT6 exon2 or 3 fusion and tumors with other fusions, with respect to tumor location in the thoracic region (P < 0.001), lower nuclear atypia/pleomorphism (P = 0.007), lower mitotic activity (P = 0.003), lower MIB-1 LI (0.019), and lower cellularity (P = 0.023). These findings suggest that an SFT with NAB2 exon4 -STAT6 exon2 or 3 is less aggressive than those without the variant. In addition, in the 3 tumors with the highly aggressive phenotype, 2 harbored NAB2 exon6 -STAT6 exon18. Statistical analysis of clinicopathologic factors between tumors with either NAB2 exon6 -STAT6 exon16/18 fusion and tumors with other fusions revealed that high mitotic account and the presence of necrosis were nearly significantly associated with the NAB2 exon6 -STAT6 exon16/18 fusion.

Genetic alterations in either of p53, PDGFRβ, or TERT promoter regions were detected in 8 cases. Mutations in p53 were detected in 2 cases, both of which were dedifferentiated SFTs only in the dedifferentiated component. Mutations of PDGFRβ were observed in 3 conventional cases, two of which are of pleural origin and one of brain origin. Mutations of TERT promoter region were found in 5 cases (2 pleural tumors and 3 soft tissue tumors). All of these mutations were C228T mutations. In addition, both dedifferentiated SFTs harbored this type of mutation, and these mutations were preserved in the conventional and dedifferentiated components of the SFTs. Interestingly, the mutations in PDGFRβ and TERT promoter region were mutually exclusive, and the mutations in PDGFRβ and p53 were also mutually exclusive. Two cases of DSFT contained mutations in both p53 and TERT promoter regions. There was no association between tumors with either NAB2 exon4 -STAT6 exon2 or 3 fusion and tumors with other fusions regarding the frequency of mutations in the examined genes.

Discussion: An SFT with the NAB2(exon4)-STAT6(exon2/3) fusion transcript was associated with less aggressive clinicopathologic features. In contrast, Two of 3 malignant SFTs harbored the NAB2(exon6)-STAT6(exon18) fusion transcript, suggesting that tumors with this fusion variant also manifest aggressive clinicopathologic phenotypes, in addition to tumors with the NAB2(exon6)-STAT6(exon16/17) variant. In this series, p53-positive staining was associated with aggressive clinicopathological features and lower disease-free survival rates in 38 cases of SFTs excluding 2 cases of DSFT. This result suggests that p53 overexpression plays a role in the acquisition of aggressive phenotype in this tumor. Furthermore, both dedifferentiated tumors showed strong and diffuse p53 expression in the dedifferentiated areas and
harbored p53 mutations only in the dedifferentiated component. In addition, two cases of DSFTs also harbored mutations in TERT promoter regions. Although the mechanisms governing dedifferentiation in SFTs remain to be fully elucidated, these findings suggest that p53 mutations that lead to its overexpression, in combination with TERT promoter hot spot mutations, may very well play an important role in the dedifferentiation process.

**Significance:** This study delineates the associations between characteristic clinicopathologic features and type of NAB2-STAT6 fusion gene, with particular focus on the impact of these secondary genetic alterations on the tumor biology - especially on the dedifferentiation process in SFTs.

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