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# Whole-exome sequencing demonstrates recurrent somatic copy number alterations and sporadic mutations in specialized stromal tumors of the prostate☆,☆☆

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**Summary** In a previous array comparative genomic hybridization study, we detected common deletions of chromosomes 13 and 14 in prostatic stromal sarcoma and stromal tumor of uncertain malignant potential (STUMP). In this study, we performed whole-exome sequencing (WES) and fluorescence in situ hybridization to explore somatic mutations in 1 low-grade stromal sarcoma, 1 high-grade stromal sarcoma, and 12 STUMPs including 5 cases of degenerative atypia type, 1 myxoid type, 1 phyllodes type, and 5 cases of recently described round cell type. WES was successful on 13 cases that revealed frequent somatic copy number alterations including losses of chromosomes 13 (11 cases), 14 (11 cases), and 1p (9 cases), and partial or complete loss of chromosome 10 (7 cases). Fluorescence in situ hybridization was done on 9 cases and showed compatible chromosome 13 copy numbers with the WES results. STUMPs and the low-grade stromal sarcoma carried moderate tumor mutation burdens that ranged from 1.23 to 7.24 mutations per megabase, while the high-grade stromal sarcoma harbored a significantly higher mutation burden (11.55 mutations per megabase). Sporadic somatic mutations were observed, but no recurrent driver mutations could be discerned. In conjunction with prior array comparative genomic hybridization, we have demonstrated the consistent gene dosage profiles that support the clonal nature and the concept of specialized stromal tumors of the prostate as a distinctive tumor entity.


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## 1. Introduction

Prostatic stromal tumors arising from the specialized prostatic stroma are rare and distinct with diverse histologic patterns [1,2]. In the past, these tumors have been reported under a variety of terms including *atypical stromal (smooth muscle) hyperplasia*, *phyllodes type of atypical stromal hyperplasia*, and *cystic epithelial-stromal tumors* [3-6]. Some of these terms indicate controversy whether these lesions are neoplastic or variants of stromal hyperplasia. A classification of these stromal lesions was later proposed, identifying (1) prostatic stromal sarcoma and (2) stromal tumor of uncertain malignant potential (STUMP) [1]. Four histologic patterns, namely, degenerative atypia pattern, hypercellular pattern, myxoid pattern, and phyllodes-type growth pattern, were recognized. Stromal sarcomas are subtyped as low grade and high grade on the basis of cellular pleomorphism and degree of cellularity [2]. Follow-up of these patients revealed unique morbidity [2]. STUMP can recur frequently, occur at a younger age, predominantly involve the peripheral zone where they can adhere to the rectum requiring its removal, and dedifferentiate to or be concurrently associated with stromal sarcoma. Low-grade stromal sarcoma can locally invade, and high-grade sarcoma has the potential to metastasize. Recently, a round cell pattern composed of hypercellular stroma with rounded cells partially displacing other glands has been described, further expanding the morphologic spectrum of STUMP [7].

Although the histologic and immunohistochemical patterns of STUMP and stromal sarcoma have been characterized, little is known about the molecular basis of these tumors. In a previous study of array comparative genomic hybridization (aCGH) [8] on a total of 11 cases, we have demonstrated common somatic copy number alteration (SCNA) including the losses of chromosomes 13 and 14. The recurrent chromosomal alterations imply the clonal nature of specialized stromal tumors of the prostate as a distinctive tumor entity rather than hyperplasia. In this multi-institutional study, we collected 14 cases including the recently reported round cell type. We performed whole-exome sequencing (WES) to detect somatic alteration and fluorescence in situ hybridization (FISH) to validate the loss of chromosome 13 in these tumors.

## 2. Materials and methods

### 2.1. Sample collection

This study was approved by the institutional review board of the Taipei Veterans General Hospital (#2015-03-005B, #2014-03-008A). The series included 7 cases of STUMP (cases 1, 6, 7, 8, 23, 24, and 25) contributed by Dr Jonathan I. Epstein (J. I. E.), 5 cases of STUMP (cases 18-22) collected by Dr Toyonori Tsuzuki from Japan, 1 low-grade stromal sarcoma (case 9) contributed by J. I. E., and 1 high-grade stromal sarcoma (case 10) from Taipei Veterans General Hospital (Table). The materials of cases 1, 6, 7, 8, 9, and 10 were used and reported in our previous study of aCGH [8]. For the cases of STUMP, cases 6, 7, and 18-20 were subtyped as degenerative atypia type, case 1 as myxoid type, case 8 as phyllodes type, and cases 21 to 25 as round cell type. Representative histology is shown in Fig. 1.

### 2.2. DNA extraction

Cases 10, 18, and 20-24 had paired tumor and normal tissue samples submitted for WES. Other cases consisted only of tumor samples for analysis. Case 25 had only unstained slides available, which were not suitable for WES. DNA was extracted from the tissue cores punched out from normal blocks and tumor blocks of formalin-fixed, paraffin-embedded specimens following QIAamp protocol (Qiagen, Foster City, CA). Because WES requires a large amount of DNA, microdissected specimens are not adequate. For tumor specimens, we selected the areas where stromal cells accounted for >80% of cell population. The quantity (OD 260 nm) and quality (OD 260 nm/OD 280 nm) of DNA in the obtained solution were measured using an ND-1000 spectrophotometer (Nanodrop Technology, Wilmington, DE). The integrity of the DNA was determined using an Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA).

### 2.3. Whole-exome sequencing

#### 2.3.1. Library construction and sequencing

We used Agilent SureSelect XT Reagent kit protocol for Illumina Hiseq paired-end sequencing library (catalog #G9611A). In all cases, the SureSelect XT Human All Exon Version 5 probe set was used. We constructed library with Agilent SureSelect XT Reagent kit. The amplification adapter-ligated sample was purified using Agencourt AMPure XP beads (Beckman Coulter, Brea, CA) and analyzed on a Bioanalyzer DNA1000 chip. A total of 500 ng of the sample DNA was prepared for the hybridization with the capture baits, and the sample was hybridized for 24 hours at 65°C, captured with the Dynabeads MyOne Streptavidin T1 (Life Technologies, Carlsbad, CA) and purified using Agencourt AMPure XP beads. All samples were sequenced on Illumina HiSeq 2000.

#### 2.3.2. Data analysis

We followed the GATK Best Practice (BWA mem alignment to hg19, Mark duplicates, Base recalibration) with standard filtering parameters and variant quality score recalibration [9,10]. We used GATK HaplotypeCaller and Varscan2 [11] to call variants. For cases with matched normal sample, we used Varscan2 somatic function to call somatic variants. For cases without normal sample, the possible germline pleomorphisms were vigorously filtered out as follows: First, we adopted the algorithm of Rizvi et al [12] with modification.